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Synthesis and Evaluation of Cocaine Analogs for Activity as Cocaine Antagonists and Synthesis of Novel Prodrugs with Antiarthritic Potential

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SYNTHESIS AND EVALUATION OF COCAINE ANALOGS FOR ACTIVITY AS COCAINE ANTAGONISTS AND SYNTHESIS OF NOVEL PRODRUGS WITH ANTIARTHRITIC POTENTIAL

by

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ABSTRACT

BONNIE LISA BARR, Synthesis and Evaluation of Cocaine Analogs for Activity as Cocaine Antagonists and Synthesis of Novel Prodrugs with Antiarthritic Potential. (Under the guidance of JAMES E. WYNN, Ph. D.)

As a follow up to interest in a new class of agents that have potential for the treatment of arthritic conditions, two series of cocaine derivatives were synthesized. Specifically, an N-alkyl series consisting of the ethyl, *n*-propyl, isopropyl, *n*-butyl and benzyl norcocaine derivatives and an N-acyl series consisting of the acetyl, propionyl, butyryl, and benzoyl norcocaine derivatives were synthesized. The structures of these compounds were verified using GC/MS, ^{1}H NMR, and FT-IR with support from elemental analysis. Evaluation of the effect of these cocaine analogs on the inhibition of dopamine uptake represented the biological focus of this project. Each of these series of compounds was evaluated in ligand binding and neurotransmitter uptake blockade assays as part of an ongoing research project at the National Institute of Drug Abuse. This program is designed to screen compounds for their usefulness in the treatment of cocaine abuse. None of the compounds in these two series demonstrated sufficient *in vitro* activity to warrant animal testing.

A separate small series of unique compounds in which the 3-carboxylic acid moiety of benzoylecgonine was reduced to a primary alcohol and esterified with various nonsteroidal antiinflammatory drugs were synthesized. These compounds are potential prodrugs for the esterified NSAID and for benzoylecgonine. They will be evaluated for potential antirheumatic activity as part of a future study.

DEDICATION

This dissertation and the research involved in it is dedicated:

To my family: My parents, Les and Ellen Barr; my sisters Robin, Cindy and her husband Todd; my brother Less; my nieces Sarah and Hannah; and my grandmother Bonnie Donihe. You tolerate, accept and support my "eccentricities" and haven't disowned me.....yet.

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BACKGROUND AND SIGNIFICANCE

Connective tissues diseases can be divided into a group of relatively uncommon, genetically determined disorders affecting the primary structure of this tissue and a number of acquired maladies where the connective tissues serve as the site of several distinctive immunologic and inflammatory reactions. These diseases are now taken to include the following: 1) rheumatoid arthritis (RA); 2) systemic lupus erythematosus; 3) progressive systemic sclerosis; 4) polymyositis and dermatomyositis; 5) Sjögren's syndrome; 6) amyloidosis; 7) necrotizing arteritis and various other forms of vasculitis and 8) rheumatic fever. (1) This overview will focus primarily on RA and its treatment. Because of related drug treatment, the condition of osteoarthritis (OA) will also be discussed.

Acquired connective tissue diseases have been classified as autoimmune disorders based on several criteria: 1) increased levels of serum immunoglobulin; 2) presence of specific immunoglobulins that react with certain serum proteins and various nuclear and cytoplasmic components of tissue, causing production of autoantibodies; 3) antigen-antibody complexes at sites of tissue damage; 4) accumulation of plasma cells and lymphocytes in affected tissue; 5) relief of symptoms with treatment of corticosteroids and/or other agents which inhibit production of autoantibodies; 6) association with other disorders known to be the result of problems with immunologic response(1).

Osteoarthritis (OA) or degenerative joint disease is extremely common. It is a noninflammatory, progressive disorder of the movable joints with a pathological characterization which includes deterioration of the articular cartilage and formation of new bone in the subchondral areas and at the margin of the joint. It may occur as an inherent part of the aging process or secondary to joint injury, dysplasia, or other types of arthritis.

The clinical features presenting during the development of degenerative joint disease include the excessive pressure on the joints associated with obesity and acquired or developmental structural abnormalities; various metabolic disturbances such as alkaptonuria and acromegaly; repeated joint hemorrhage; disordered proprioceptive sense and a genetic predisposition for certain forms of the disease(1).

Common complaints from patients with OA are joint pain, particularly on motion and weight-bearing joints; stiffness after periods of rest and aching in times of inclement weather. Physical examination reveals crepitation on joint motion, spasm or atrophy of the surrounding muscles, decreased range of motion, malalignment of the extremity, and changes in the joint shape.

Laboratory findings in OA are very much different from those associated with RA. Hematocrit, white blood cell count, and serum protein electrophoresis are usually normal. Erythrocyte sedimentation rate (ESR) is also normal while the latex agglutination test for rheumatoid factor (RF), a high molecular weight protein (IgM) found in increased levels in the serum of patients with rheumatoid arthritis, is negative. Synovial fluid analysis reveals little or no sign of inflammation(1).

Treatment objectives are relief of pain, restoration of function of the joint, and/or

prevention of avoidable disability or progression of the disease. Physical measures include daily rest periods with support of the affected joints. Correction of abnormal posture, avoidance of unnecessary walking and stair-climbing are advisable. Local measures include heat and exercises, while traction may be useful when there is muscle spasm.

Drug therapy plays a relatively minor role on the treatment of OA except for the use of analgesics. Aspirin (600 mg three to five times daily) may be useful in conjunction with the measures listed above. Agents with greater toxicity are rarely warranted since they would have to be used on a long-term basis.

Often surgical intervention is necessary for the relief of persistent pain and correction of severe deformity. The most common surgical procedures are debridement, arthrodesis, arthroplasty, osteotomy, and total joint replacement(1).

Rheumatoid arthritis is a chronic disease characterized by nonsuppurative inflammation of the diarthrodial (synovial) joints frequently combined with extra-articular manifestations such as vasculitis, pulmonary nodules, and chronic leg ulcers. Other symptoms include inflammation of the synovium accompanied by edema, vascular congestion, fibrin exudate, cellular infiltrate, and increased synovial fluid with increased turbidity. While the extent of the disease varies from patient to patient, it is characterized by a tendency toward spontaneous remission and exacerbation (1).

The degree of articular disability depends mainly on the amount of damage done to the cartilage. Severe injury leads to denudation of large areas of bone and formation of adhesions between the joint surfaces with subsequent transformation of these adhesions

into bony connective tissue which can lead to total immobility of the joint.

Physical evaluation of the disease in the early stages is crucial to eliminate other diagnostic possibilities and to begin a treatment regimen that will encourage improvement or even remission of the condition. The diagnosis is established by a typical clinical picture, histopathologic features, and certain characteristic laboratory findings such as anemia, elevated ESR, and hypergammaglobulinemia. A latex agglutination test reveals presence of rheumatoid factor.

In 1987, a subcommittee of the American College of Rheumatology formulated the following set of criteria for the classification of RA shown in Table 1. A patient is diagnosed with RA if at least four of the seven criteria apply(2). These criteria must persist for at least 6 weeks.

Although some patients are afflicted with a mild form of the disease that may last a relatively short time and cause little or no permanent damage, many others suffer from a recurrent or sustained condition causing a serious and permanent disturbance in joint function. At sites of involvement, swollen, boggy, joints occur as the result of intra-articular effusion, edema at periarticular structures, and the overgrowth of the hyperplastic synovial membrane and variable degrees of periarticular fibrosis. Poor prognosis with respect to joint function include persistent disease greater than one year duration, age of onset less than thirty, sustained disease, and high RF titers.

Table 1

Criteria For The Classification Of Rheumatoid Arthritis

Criterion	Definition
Morning Stiffness	*Morning stiffness in and around the joints lasting at least one hour before maximal improvement.*
Arthritis of three or more areas	*At least 3 joints have had soft-tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible joint areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints.**
Arthritis of hand joints	*At least one area swollen (as defined above) in a wrist, MCP, or PIP.*
Symmetric arthritis	*Simultaneous involvement of the same joint areas on both sides of the body (bilateral involvement of PIP, MCPs,or MTPs is acceptable without absolute symmetry).*
Rheumatoid nodules	*Subcutaneous nodules, over bony prominences, extensor surfaces, or juxtaarticular regions observed by a physician.*
Serum rheumatoid factor	*Demonstration of abnormal amounts of serum RF by any method that has been positive in less than 5% of normal controls subjects.*
Roentgenographic changes	*Roentgenographic changes typical of RA on AP hand and wrist roentgenograms, which must include erosions or unequivocal bony decalcification localized to or most marked adjacent to the involved joints (osteoarthrosis changes alone do not apply)*

* PIPs, proximal interphalangeal joints; MCPs, metacarpophalangeal joints; MTPs, metatarsophalangeal joints; AP, anteroposterior

Rheumatoid arthritis is chiefly a disease of the joints, but often there are systemic manifestations involved. These features are apparently the result of underlying vasculitis

more commonly observed in patients with subcutaneous nodules and high RF titers. They include nodules in the lungs and the scleral coat of the eye, various nerve compression syndromes, ischemic ulcers characteristically located distally on the leg, and normocytic, slightly hypochromic anemia.

The treatment regimen for a patient suffering from RA should be determined by the following factors: 1) the status of joint function, especially range of motion, 2) degree of disease activity, 3) the age, sex, occupation, and family responsibilities of the patient and his/her response to the disease, and 4) the results of previous treatment(1).

The drug classes used the treatment of RA are as follows: 1) nonsteroidal anti-inflammatory drug (NSAIDs), 2) immunosuppressive agents, 3) immunostimulatory drugs, and 4) disease modifying antirheumatic drugs (DMARDs). The type drug used is determined by the above criteria.

The NSAIDs were initially developed as antipyretics. These drugs have survived to the present because they were found to be excellent analgesics for the relief of minor aches and pains. At lower doses, they act as simple analgesics, but at higher levels they have an effect on the other features of inflammation including the interruption of the arachidonic acid cascade by prostaglandin synthetase or cyclo-oxygenase inhibition(3,4).

Inflammation is a complex reaction to an insult to the tissue. Following cellular damage, chemical mediators such as histamine, bradykinin, 5-hydroxytryptamine, slow-reacting substance of anaphylaxis (SRS-A), chemotactic factors and prostaglandins are released locally. Phagocytic cells migrate into the affected area and contribute to the reaction by releasing lytic enzymes. The ability of NSAIDs to inhibit the production of

prostaglandins has been thought to account for their anti-inflammatory effects, but various mechanisms have been proposed including interference with cellular metabolism, release of inflammatory mediators, sodium and potassium ion transfers across cellular membranes, inhibition of chemical mediators other than prostaglandins, and stabilization of lysosomes (4).

Salicylic Acid Derivatives:

Aspirin (acetylsalicylic acid) (ASA)(Fig. 1) is the prototype compound in the salicylate category of the NSAIDs. Salicylates generally act by virtue of their salicylic acid content, although some effects of ASA are due to its capacity to acetylate proteins. Substitutions on the carboxyl or hydroxyl groups change the potency or toxicity of the compound. The *ortho* position of the hydroxyl group is important in the action of salicylates. Benzoic acid has many of the actions of salicylic acid while being much weaker. The effects of simple substitutions have been extensively studied and new salicylates are being synthesized (3).

Salicylates are regarded as the standard by which other drugs are compared in the treatment of RA. In addition to the obvious benefits of analgesia, there is shown to be an improvement in appetite and an overall feeling of well-being. They also reduce the inflammation in joint tissues and surrounding structures when given in large doses for long periods to patients with active rheumatoid disease. These doses average from four to six grams daily, although some patients respond to less. Aspirin is thought to work specifically by inhibiting cyclo-oxygenase in the arachidonic acid cascade, causing inhibition of prostaglandin synthesis. Other salicylates have similar mechanisms of

action.

The major toxicity with the high dose salicylate therapy needed to treat RA is the gastrointestinal distress and ulcerogenic properties of these compounds. Exacerbation of peptic ulcer symptoms, gastrointestinal hemorrhage, and erosive gastritis have all been reported. The gastric bleeding occurs in highest frequency with the use of salicylates that dissolve slowly and deposit as particles on the gastric mucosa. This problem may be avoided with the use of enteric coated products. The acute gastric irritancy of ASA may be associated with the carboxylic acid group. Modifications of the acidic characteristics of this group (perhaps by esterification) might also be a way to decrease the irritancy while retaining anti-inflammatory activity(5). For example, introduction of a 3-methyl group (adjacent to the phenolic OH group) in ASA and salicylic acid produces 3-methyl acetylsalicylic acid and 3-methyl salicylic acid, which displays a slower metabolic excretion. The 3-methyl group slows hydrolysis of the acetyl group and formation of the metabolite, 3-methyl salicylic acid. Aspirin derivatives modified on position 5 show greater antiinflammatory activity than ASA.

Hepatotoxicity has also been seen in patients being treated for connective tissue disorders. Most patients are asymptomatic, with elevated hepatic enzymes the primary indicators of tissue damage. If symptoms do occur (such as hepatomegaly, anorexia, nausea, and jaundice), the salicylates should be discontinued due to the potential for fatal necrosis.

Diflunisal (Dolobid®)(Fig.1), 5-(2,4-difluorophenyl)salicylic acid is more potent than ASA with no apparent effect on blood platelet aggregation and is reported not to cause

gastric bleeding. It is believed to inhibit production of prostaglandins (5).

5-Pyrazolones:

The 5-pyrazolones are important because they led to the potent phenylbutazone class of NSAIDs. The anti-inflammatory activity of phenylbutazone (Butazolidin®)(Fig. 1) is somewhat less potent than that of indomethacin but greater than ASA. This activity is not mediated by the adrenal cortex, but more likely by peripheral mechanisms involving a decrease in capillary permeability. Phenylbutazone shows the same biochemical reactions as the other NSAIDs. Some of the mechanisms of action for ASA may also apply to phenylbutazone including uncoupling of oxidative phosphorylation, inhibition of prostaglandin synthesis, inhibition of the histamine-forming enzyme histidine decarboxylase, stabilization of lysosomal membranes, and inhibition of mucopolysaccharide biosynthesis.

The therapeutic action of phenylbutazone is characterized by relief of pain, reduction of fever, and decrease in swelling, tenderness, and local heat. The initial daily dose for RA is 300 to 600 mg. divided into three to four doses. The drug has pronounced toxicity and should not be used for periods exceeding one week.

Many modifications of the phenylbutazone structure have been made in attempts to increase activity and decrease toxicity. Because of the close relationship between the acidity and biologic activity of these derivatives, increasing the acidity will decrease the anti-inflammatory activity and sodium-retaining potency, but will greatly increase the uricosuric effects(5).

Phenylbutazone and the anti-inflammatory 3,5-pyrazolidinediones, like the salicylates,

possess ionizable protons (here enolic or potentially enolic). The process of ionization for the acidic protons in carboxylic and enolic acids may be compared as follows. Replacement of the hydrogen atom at C-4 of phenylbutazone by a CH_3 to give 4-butyl-4-methyl-1,2-diphenyl-3,5-pyrazolidinedione destroys anti-inflammatory activity. This suggests that the enolizable *beta*-dicarbonyl system is essential. The butyl group at C-4 may be replaced by a propyl group or allyl group without decreasing the anti-inflammatory activity. The presence of a keto group in the *gamma* position of the butyl side chain produces the active compound, *gamma*-ketophenylbutazone.

Activity is also retained when the *para* position of one or both benzene rings carries such substituents as methyl, chloro, or nitro groups. Oxyphenylbutazone, a metabolite of phenylbutazone, contains a *para*-hydroxy group on one of these rings and is a potent anti-inflammatory agent.

Replacement of one of the nitrogen atoms in the pyrazolidine nucleus with an oxygen yields an isoxazole analog with comparable anti-inflammatory activity. Compounds in which a cyclopentane or cyclopentene ring replace the pyrazolidine ring are inactive.

Indomethacin:

The aryl- and heteroarylacetic acid derivatives show high analgesic potency as well as anti-inflammatory activity. The prototype drug in this class is indomethacin (Indocin®). (Fig. 1) The mechanism of action may be polyvalent and similar to the other acidic anti-inflammatory agents. These effects are not a result of stimulation of the production or release of corticosteroids. Indomethacin benefits RA patients by relieving pain, decreasing swelling and tenderness of the joints and increasing grip strength.

A systematic variation of the indole ring substituents on of indomethacin reveals the structure activity relationship (SAR) of this class of drugs. The COOH is necessary for anti-inflammatory activity since replacement with other groups decreases this activity. In addition, the more acidic the COOH group, the greater the potency of antirheumatic activity. Replacement of the 1-arylacyl group by an arylalkyl function decreases activity, as does an aliphatic acyl or alkyl substitution at that position. A *para* halogen or equivalent (CF_3 or SCH_3) substituted for the 1-benzoyl group provides the greatest activity.

At the 2 position, a methyl group yields greater activity than an aryl group. At the *alpha* position on the side chain, a hydrogen and a methyl are in essence equal but an *alpha, alpha*-dimethyl or hydroxyl functional group decreases the potency. Position 5 on the ring is most favorable for substitution. Methoxy, allyloxy, dimethylamino, acetyl, methyl, and fluoride functions show greater activity to hydrogen and chloride.

In the indomethacin molecule, the 3-acetic acid side chain undergoes free rotation assuming different conformations. Of the *alpha*-methyl acetic acid analogs, anti-inflammatory activity is displayed only by dextrorotatory enantiomers, which were later shown to have sinister (S) absolute configuration.

The *para*-chlorophenyl group at N-1 is also free to rotate and assume different conformations. The preferred isomer has the *p*-chlorophenyl group *cis* to the methoxyphenyl portion of the indole ring, yet it is not planar with the indole nucleus. This lack of planarity is due to the steric hindrance created by the 2-methyl group (necessary for high activity) and the hydrogen atom at position 7 which forces the *para*-

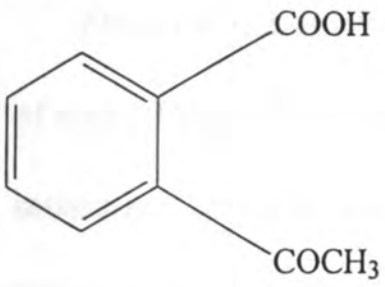
chlorophenyl group out of the plane of the ring. The usual dose is 75 mg. daily or twice daily(5).

Sulindac:

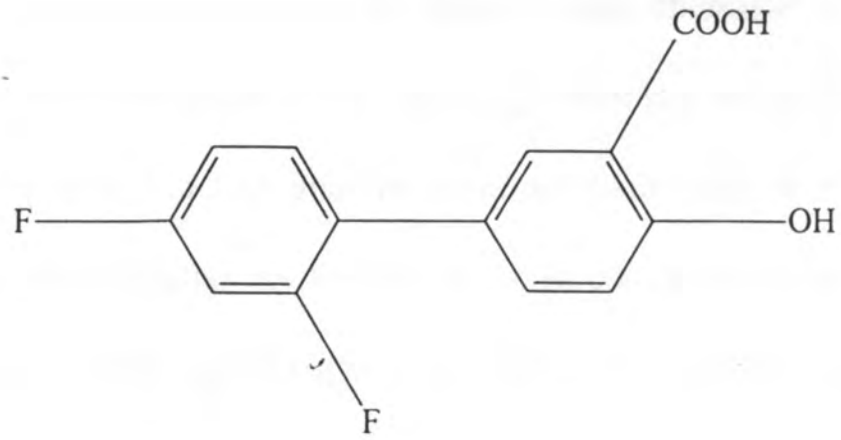
The SAR studies of indomethacin and the 1-arylindenyl isostere aided in the development of sulindac (Clinoril®)(Fig. 1). The heterocyclic nitrogen atom was removed resulting in an indene isostere that is an active compound lacking CNS activity and having less GI irritancy than indomethacin. Since this compound is insoluble in urine, further modification was necessary to allow clinical use. Substitution of a fluoride and methylsulfinyl group increases the solubility while retaining the potency. The anti-rheumatic oral dose is 150 to 200 mg twice daily.

Diclofenac:

One of the most widely used NSAIDs, diclofenac (Voltaren®), possesses structural characteristics of both arylalkanoic acid and anthranilic acid classes of anti-inflammatory agents(Fig.2). It displays anti-inflammatory, analgesic, and antipyretic properties. It has three possible mechanisms of action: 1) inhibition of the arachidonic acid cyclooxygenase system resulting in decreased production of prostaglandins and thromboxanes; 2) inhibition of the lipooxygenase pathway which decreases production of leukotrienes; and 3) inhibition of arachidonic acid release and stimulation of its reuptake thereby decreasing arachidonic acid availability. It is indicated for the treatment of RA, OA, and ankylosing spondylitis in doses ranging from 100-200 mg/day.



aspirin



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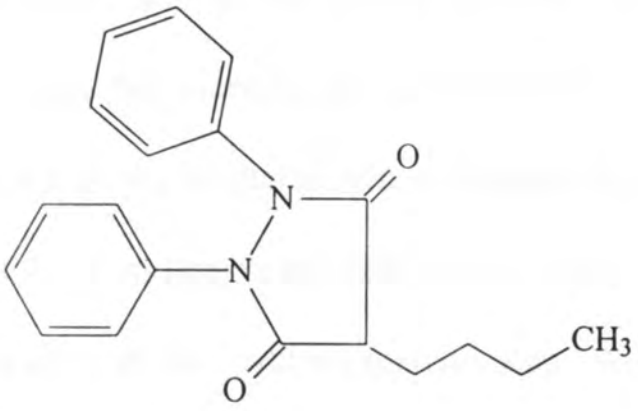
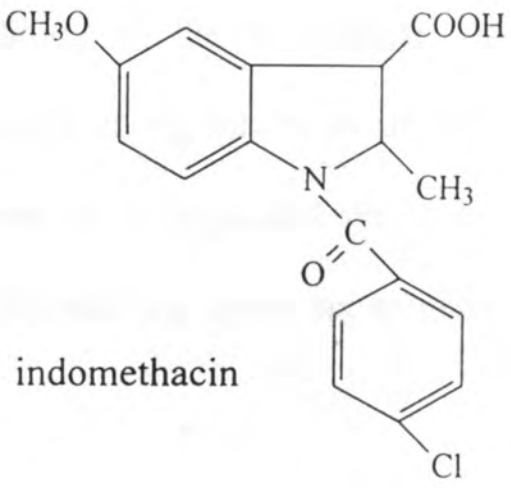
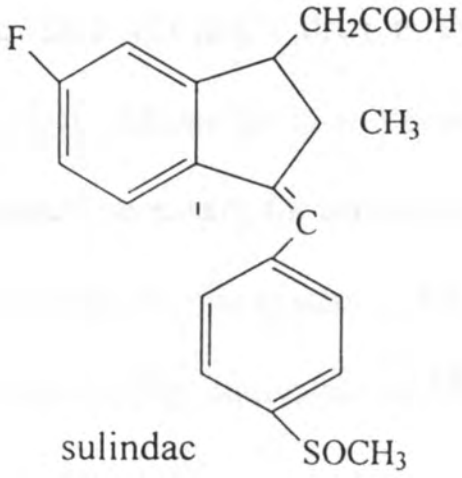


phenylbutazone



indomethacin



sulindac

Fig. 1

Etodolac:

Etodolac (Lodine®)(Fig. 2) has been promoted at the first in a new chemical class of anti-inflammatory agents, the pyranocarboxylic acids. Although there is a two carbon separation between the carboxylic acid function and the heteroaromatic ring, it still displays the structural characteristics similar to the hetero-arylacetic acids. It is recommended for the management of OA, and for use as an analgesic. Etodolac also possesses some antipyretic activity as well. At 2.5 to 3 times the effective dose, it produces less GI bleeding than indomethacin, ibuprofen, or naprosyn, giving this compound a potential therapeutic advantage in patients prone to GI distress.

Etodolac's primary mechanism of action is inhibition of the boisynthesis of prostaglandins at the cyclooxygenase step, with no accompanying inhibition of the lipoxygenase system. It is recommended for the treatment of the signs and symptoms of OA (800-1200 mg/day) and for pain management (200-400 mg every six to eight hours as needed).

Tolmetin:

Tolmetin (Tolectin®)(Fig. 2) is the major drug in the pyrrolacetic acid derivative class. It is similar in structure to indomethacin and contains the three moieties of the indomethacin molecule considered necessary for activity: the carboxyl function, an out-of-plane phenyl ring, and the flat indole ring system. The latter was replaced in tolmetin by a pyrrole ring. The dose of tolmetin equivalent to 4000 mg of ASA in RA is 1260 mg daily (5).

Phenyl-*alpha*-methylacetic acids:

The phenyl-*alpha*-methylacetic acid derivatives are a widely used class of anti-inflammatory agents. The most commonly prescribed drugs in this category are ibuprofen (Motrin®), fenoprofen (Nalfon®), ketoprofen (Orudis®), flurbiprofen (Ansaid®), ketrolac (Toradol®), and oxaprozin (Daypro®). These drugs are chemically related to indomethacin, tolmetin, and naproxen.

Ibuprofen (Fig. 2) also inhibits prostaglandin formation. It may also cause less gastric distress than ASA. The *alpha*-methyl group on the acetic acid moiety causes an increase in potency. The antiinflammatory activity is found exclusively in the (S)-(+) isomer; this relationship is maintained throughout the arylacetic acid series. These isomers are the more potent inhibitors of prostaglandin synthetase. A dose of 1600 to 2400 mg per day is as effective as ASA in RA (5).

Fenoprofen (Fig. 2) differs from ibuprofen only in containing an *meta*-phenoxy rather than the *para*-isobutyl group In addition to its inhibition of prostaglandin synthesis, fenoprofen inhibits the rate of collagen-induced platelet aggregation, shows *in vitro* activation of the fibrinolytic system, stabilizes lysosomal membranes, and inhibits phagocytosis and complement functions (5).

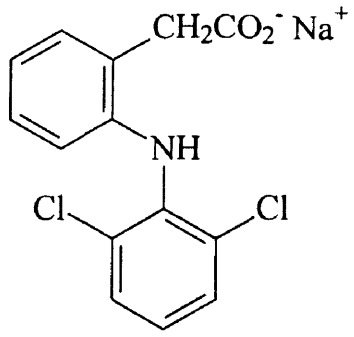
The isomer of fenoprofen in which the phenoxy group is *meta* to the propionic acid moiety is more active than either the *ortho* or *para* isomers. Optimum separation of the COOH from the aromatic group is one methylene unit. *Alpha*-substitution of the acetic acid group usually increases potency, with the methyl group (fenoprofen) being highest and the *n*-butyl group being nearly equivalent to the unsubstituted acid. *Alpha*-

substitution with any branched alkyl chain is detrimental. Ester, amide, amine, and alcohol analogs are all relatively potent, possibly by conversion to fenoprofen *in vivo*. Although fenoprofen has been resolved into its enantiomers and apparently has no significant anti-inflammatory or analgesic activity differences between either isomer or the racemic mixture. This may, however, be due to a rapid inversion of the (R)-(-) to the (S)-(+) isomer. This conversion has been shown to occur in humans. The (S)-(+) isomer has been shown to be 35 times more potent *in vivo* than the other isomer for inhibition of the cyclooxygenase pathway. For treatment of RA, the recommended dosage of fenoprofen is 300 to 600 mg three to four times daily (5).

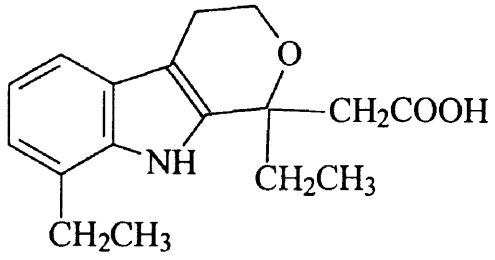
Ketoprofen (Orudis®)(Fig. 2) inhibits the synthesis of leukotrienes and leucocyte migration into inflamed joints in addition to inhibiting the biosynthesis of prostaglandins. By stabilizing the lysosomal membrane during inflammation, it decreases tissue destruction. Antibradykinin activity has also been observed with ketoprofen. This drug is indicated for the long-term management of RA and OA (in doses of 150-300 mg/day), mild to moderate pain, and primary dysmenorrhea.

Other carboxylic acids and derivatives:

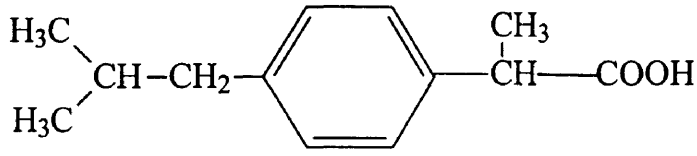
During screening of the pharmacologic properties of substituted phenylalkanoic acids, the most potent were found to be the substituted 2-(4-biphenyl)propionic acids. More detailed studies indicated that flurbiprofen (Ansaid®) (Fig. 2) had the most desirable therapeutic profile. An inhibitor of PG synthesis, flurbiprofen has anti-inflammatory activity 536 times more potent than ASA in studies done on adrenalectomized rats. It is indicated for the treatment of RA or OA in doses ranging from 200-300 mg/day.



diclofenac sodium



etodolac



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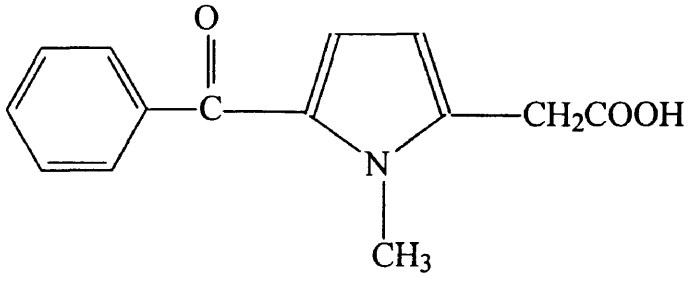

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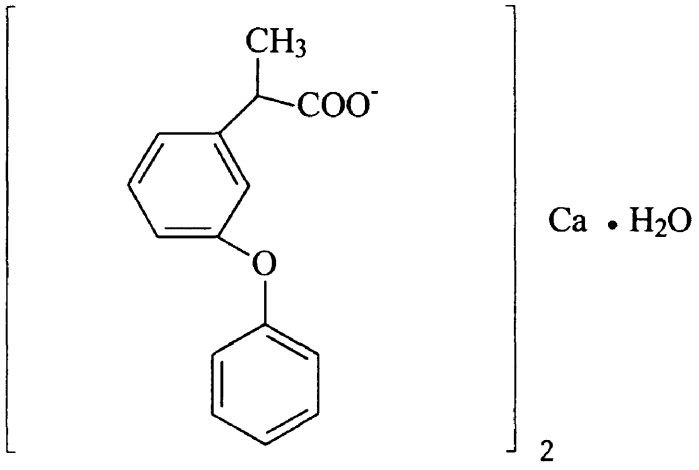

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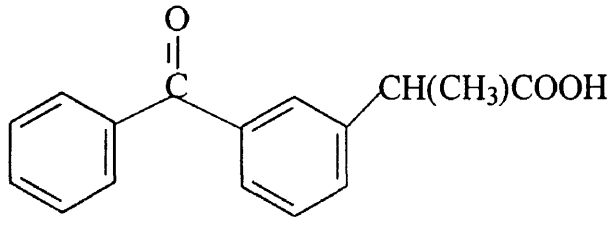

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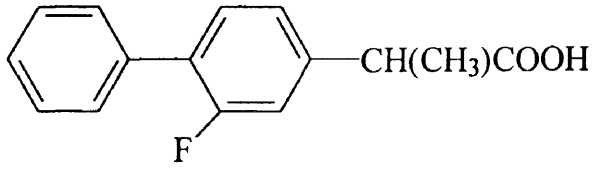


flurbiprofen

Fig. 2

Ketrolac (Toradol®)(Fig. 3), a prostaglandin synthesis inhibitor, represents a cyclized heteroaryl propionic acid with the *alpha*-methyl group fused to a pyrrole ring. It is indicated only as a peripheral analgesic although it possesses anti-inflammatory and antipyretic activity as well. A 15 to 30 mg dose of ketrolac produces analgesia equivalent to a 12 mg dose of morphine, and has therefore become an alternative to opioid analgesia. It is not indicated for the treatment of rheumatic conditions due to the often severe GI distress that may occur with oral dosing.

Oxaprozin (Daypro®)(Fig. 3), although not formally a propionic acid derivative of the *alpha*-methylacetic acid type, is similar to that class of NSAIDs and will be discussed as such. A prostaglandin synthesis inhibitor, oxaprozin exhibits antipyretic, analgesic, and anti-inflammatory acitvity. It was approved by the FDA in 1992 for the short- and long-term treatment of RA and OA with a recommended dose of 1200 mg/day.

Naproxen (Naprosyn®) (Fig. 3), the main compound in the napthyleneacetic acid derivative group, is designed to include the chemical features found in the pre-existing anti-inflammatory drugs. These are an aromatic group, and acidic group, and a side chain. It does not contain the nitrogen atom associated with some of the side effects. Modification of the side chain on the napthylene ring has shown that the 6 position was the most active. Also when the side chain is larger than OCH_3 or SCH_3, activity is reduced. The COOH group may be replaced by a hydroxyl or aldehyde group and retain function. The recommended initial dose is 250 mg twice daily. Daily doses exceeding 750 mg are not recommended(5).

N-arylanthranilic acids:

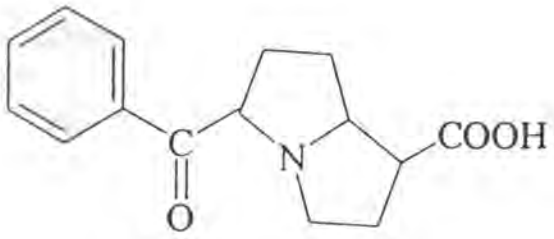
A new class of NSAIDs was developed utilizing bioisosterism. The N-arylanthranilic acids (fenamic acids) are nitrogen isosteres of salicylic acid. Their anti-inflammatory activity and analgesic properties show little advantage over the salicylates and interest in their development has diminished. Only two of these compounds, mefenamic acid (Ponstel®)(Fig. 3), used primarily for analgesia, and meclofenamate (Meclomen®) (Fig.3) are marketed in the United States. Meclofenamate is dispensed in a dosage of 200-5400 mg/day in divided doses for the treatment of RA and OA.

Oxicams:

The oxicams are a relatively new class of NSAIDs that are not carboxylic acids. These compounds are exemplified by piroxicam (Feldene®) (Fig. 3). It is a potent inhibitor of prostaglandin biosynthesis through inhibition of the cyclooxygenase step of arachidonic acid metabolism, a mechanism of action similar to other NSAIDS.

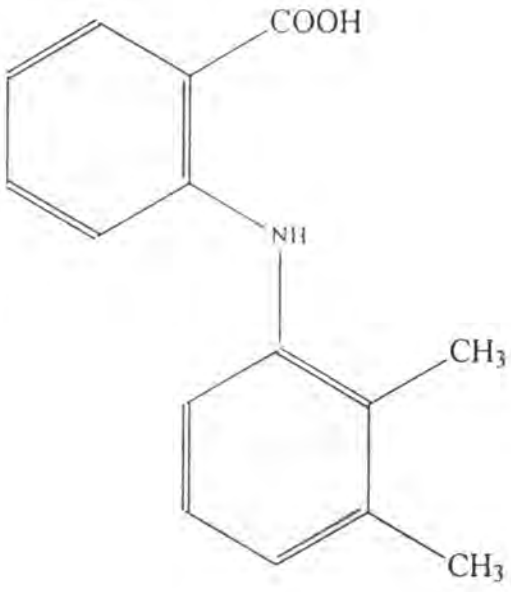
Piroxicam can cause severe GI distress. Peptic ulceration and GI hemorrhages have been reported. The recommended dosage for RA is 20 mg daily or 10 mg twice daily.

The 3-carboxamide substituent on the 1,2-benzothiazine nucleus contributes to good anti-inflammatory activity of the oxicams, as does the 2-methyl group (5).

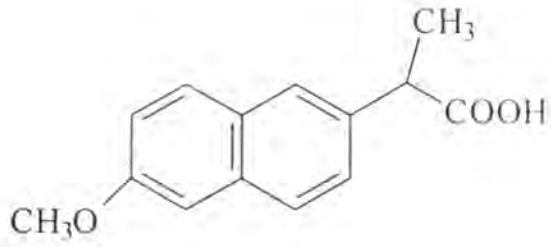


ketrolac

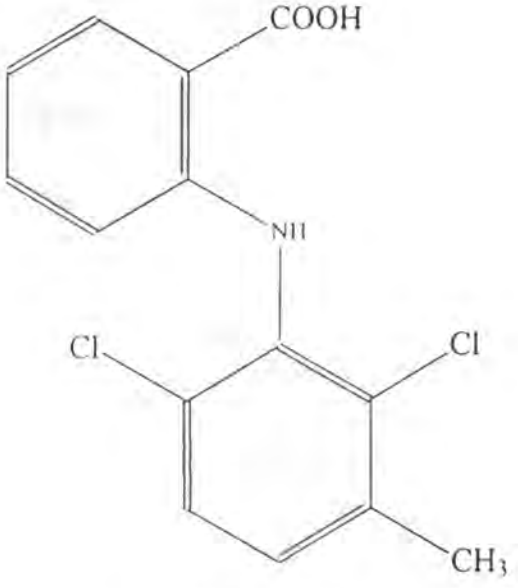
oxaprozin



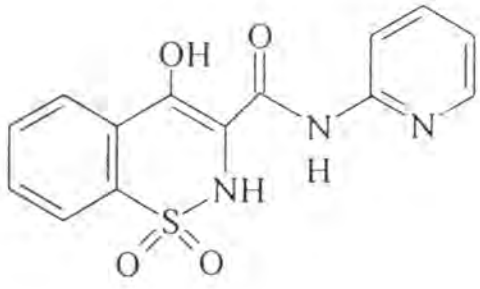
mefenamic acid



naproxen



meclomenamate



piroxicam

Fig. 3

Disease Modifying Antirheumatic Drugs

Disease modifying antirheumatic drugs (DMARDs) are a heterogeneous group of second-line agents used in the treatment of RA. They are remission-inducing agents or slow-acting anti-inflammatory medications. For the most part, these were initially developed for other disease processes and later applied to RA. In short-term randomized trials, second-line agents are more effective than NSAID therapy only, and modify the symptoms of disease activity, but do not necessarily affect a cure (6). DMARDs also lack immediate analgesic or anti-inflammatory effects and can possibly cause severe adverse effects. They can take weeks to months to produce results and are usually given concurrently with a faster-acting NSAID.

4-Aminoquinolines:

The 4-aminoquinoline antimalarial agents have been used since 1951 to treat RA. Hydroxychloroquine (Plaquenil®)(Fig. 4) is indicated in the treatment of acute and chronic RA in patients with disease states refractive to other less toxic antirrheumatics. A daily oral dose of up to 400 mg has been found to be effective (5). Clinical response can be anticipated within three to six months after initiating therapy with 40 to 60% of patients able to expect a beneficial response. Patients with earlier and milder disease have the greatest potential for a positive response on antimalarial therapy. The most common side effect is GI intolerance as signified by nausea, epigastric discomfort, anorexia, and vomiting. Infrequently, headaches, dizziness, and mental confusion may occur. Dermatologic complications are rare, but cases of exfoliative dermatitis have been seen. Neuromyopathy may occur with therapeutic doses and appears as proximal

myopathy, neuromyopathy, or cardiomyopathy. Corneal deposits occur in up to 50% of patients. These do not generally cause visual impairment, but are reversible and not a contraindication to sustained therapy. Routine (every 3 months) exams are recommended to detect early ophthalmological changes.

Gold Compounds:

The gold compounds are often considered the therapy of choice when a drug must be added to the ASA regimen. Gold compounds are currently used in the treatment of early, active RA that progresses despite therapy with aspirin-like drugs.

Three gold compounds in major use are water-soluble gold sodium thiomalate and aurothioglucose (Solgaral®)(Fig. 4), which are administered intramuscularly; and orally administered, lipid-soluble auranofin (Ridaura®)(Fig. 4). These drugs have several possible mechanisms of action. Aurothiomalate inhibits lysosomal enzymes, possibly by a reversible binding to sulfhydryl groups. Gold compounds also alter the properties of collagen in rats. *In vitro,* they inhibit prostaglandin synthesis (5).

Therapeutic response occurs in approximately 40 to 60% of patients, although 20 to 30 weeks of treatment may be necessary before relief is clinically perceptible. Parenteral gold is administered weekly at 50 mg/week for 20 to 30 weeks. Once clinical response is noted, the injections are decrease to once every three weeks, then once every two weeks, and finally monthly to arrive at a maintenance regimen of approximately 25 to 50 mg given every three to four weeks. Concurrent administration of salicylates or other NSAID is necessary during the first few months of gold therapy to provide symptomatic relief.

The adult oral dosage of auranofin is 6 mg daily. If no response is seen after six months, the dose may be increased to 9 mg daily. If still no response seen after three months at the higher dose, the drug should be discontinued (5). The onset of action for oral gold is three to nine months.

Adverse experiences occur in about 40 % of patients using IM gold. Mucocutaneous reactions and stomatitis are most common. Proteinuria and nephrotic syndrome, due to development of membranous glomerular nephropathy, may occur. Of hematologic toxicities with IM gold, eosinophilia is fairly common, and agranulocytosis or aplastic anemia, although uncommon, can be life-threatening (6). Regular urine and blood tests for renal and hematologic abnormalities are recommended. Oral gold is primarily excreted through the GI tract, and because of this, the main side effect is diarrhea. Skin rashes, hematologic toxicity and proteinuria are also seen occasionally (6). The chelating agents such as dimercaprol or penicillamine have been used to enhance excretion and control the toxic effects of these compounds (5).

Penicillamine:

Penicillamine (Cuprimine®)(Fig. 4) is indicated in patients with severe, active RA who have not responded to other therapy. It is similar to the gold compounds and may retard the progression of bone erosions. The mechanism of action is not known but may involve improvement of lymphocytic function. Penicillamine is neither anti-inflammatory nor cytotoxic. The drug should be given on an empty stomach and at least one hour apart from any other medication, food, or milk to maximize absorption and decrease the chance of inactivation by metal binding (5). The onset of action may be delayed as much

as six to nine months. The maximum dose for RA is 750 mg to 1000 mg daily. Low doses (125-250 mg) of the drug are used initially and slow-dose titration done to decrease the potential for side effects.

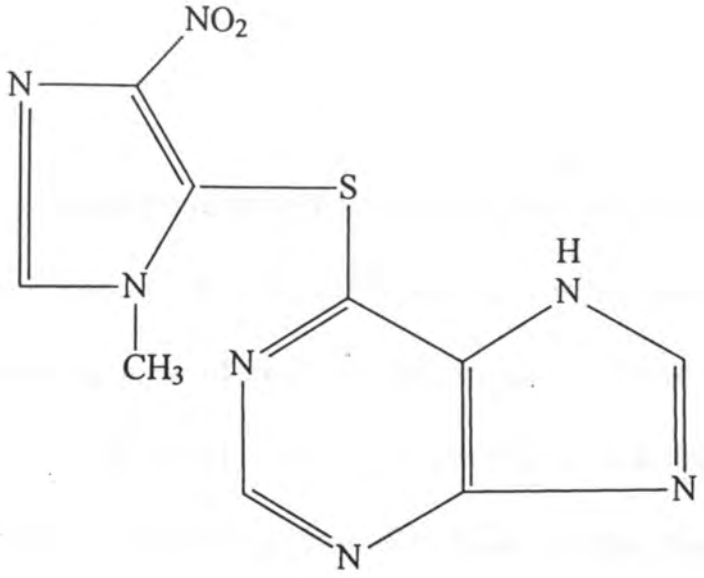
Penicillamine exhibits a toxicity profile similar to IM gold, including mucocutaneous reactions, proteinuria, nephrotic syndrome, leukopenia, thrombocytopenia, and in rare cases, aplastic anemia.

Azathioprine:

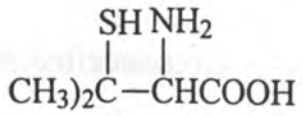
Azathioprine (Imuran®)(Fig. 4), a purine analog, prevents the conversion of purine bases by suppressing the first step of *de novo* biosynthesis of guanine and adenosine. It is the most commonly used immunosuppressive drug in RA (7). Although the mechanism of action is not understood, azathioprine has been shown to be effective at doses of 1.0 to 2.5 mg/kg/day. The onset of action is 12 to 24 weeks. The most common toxicity associated with the drug is gastrointestinal, including nausea, anorexia, and rarely, vomiting and pancreatitis. Hematologic toxicity including leukopenia, thrombocytopenia, and anemia may occur and are generally dose dependent (6).

Methotrexate:

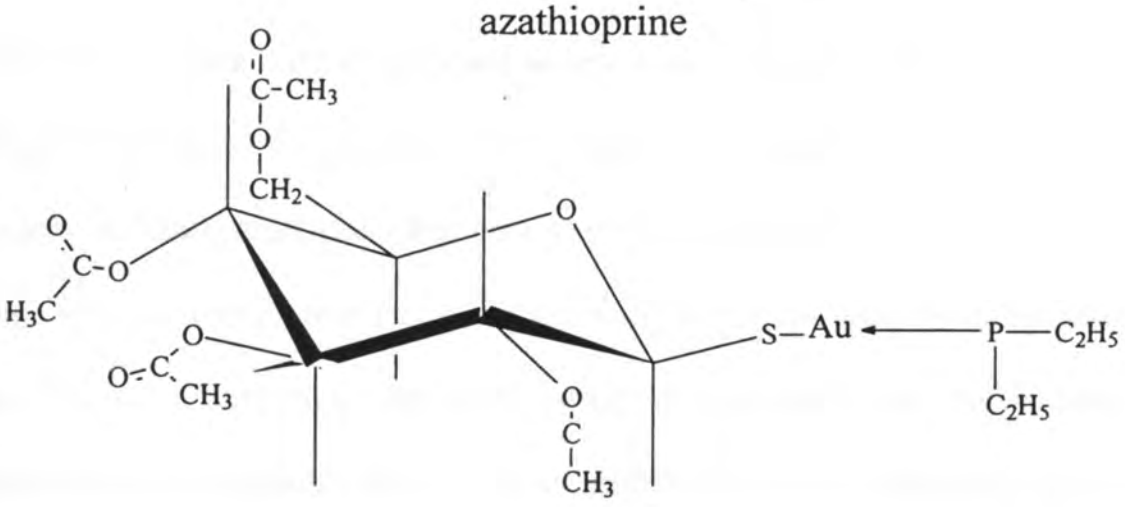
Methotrexate (MTX) (Rheumatrex®)(Fig. 5), an antifolate drug, has gained wide acceptance in the treatment of RA. It is used primarily in patients that have failed to respond to gold therapy; however, there is an increased interest in using MTX earlier in the disease prior to gold therapy. The mechanism of action of MTX in relation to RA is not known; studies have reported conflicting data. Peripheral lymphocyte phenotypes



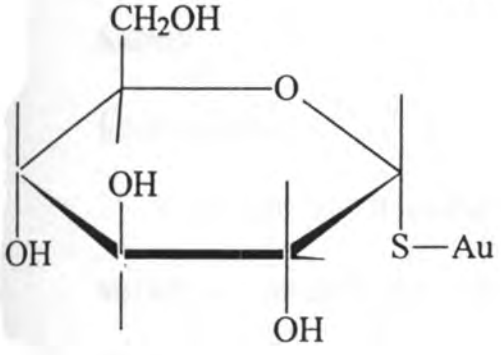
azathioprine



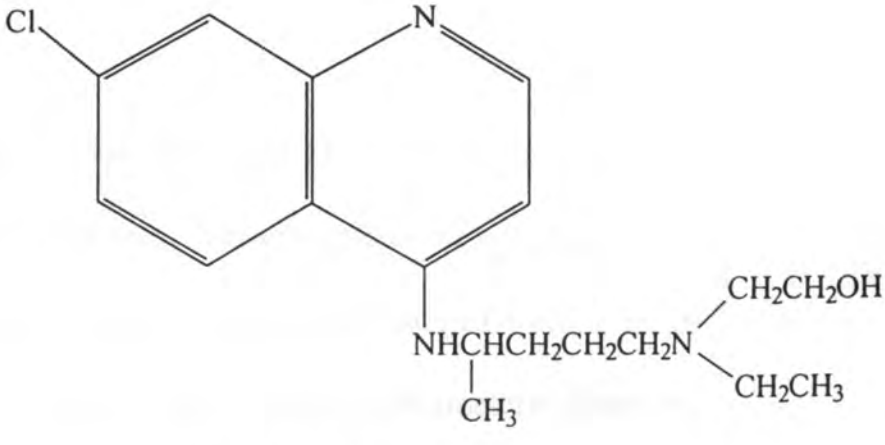
D-penicillamine



auranofin



aurothioglucose



hydroxychloroquine

Fig. 4

and *in vitro* responses to mitogen and antigen proliferation have not demonstrated global immunosuppression. *In vitro* RF synthesis was slowed by MTX therapy. Possibly a more promising area of research involves the effects of MTX on proinflammatory mediators. A consistently significant decrease in leukotriene B_4 release from stimulated WBCs *ex vivo* has been reported after MTX therapy (6).

The most common adverse reaction is GI intolerance, *e.g.* anorexia, nausea, vomiting, diarrhea, and unexplained weight loss. Stomatitis may also occur as well as alopecia, erythema in response to UV light, and urticaria. Central nervous system toxicity including headaches, dizziness, depression, and mood alteration have been noted. Bone marrow toxicity may occur and is usually associated with these risk factors: renal insufficiency, folic acid depletion, drug overdosages, and concomitant use of sulfamethoxazole/trimethoprim. Renal insufficiency of a concurrent or pre-existing nature may lead to toxic levels of the drug, because MTX is excreted exclusively by the kidney.

Cyclosporine:

Cyclosporine A (Sandimmune®)(Fig. 5) has been used as a therapeutic agent in a variety of autoimmune and inflammatory illnesses. Various studies have been done at different dosages to determine its efficacy in RA. The dosages ranged from 1 to 10 mg/kg/day. All studies showed marked improvement in disease parameters; however, side effects were significant in each. These include hypertension, fatigue, thrombocytopenia, significant renal dysfunction (*e.g.* increased serum creatinine), GI intolerance, and skin rash. The renal toxicity in particular limits the therapeutic

applications of cyclosporine A in nontransplant situations (6).

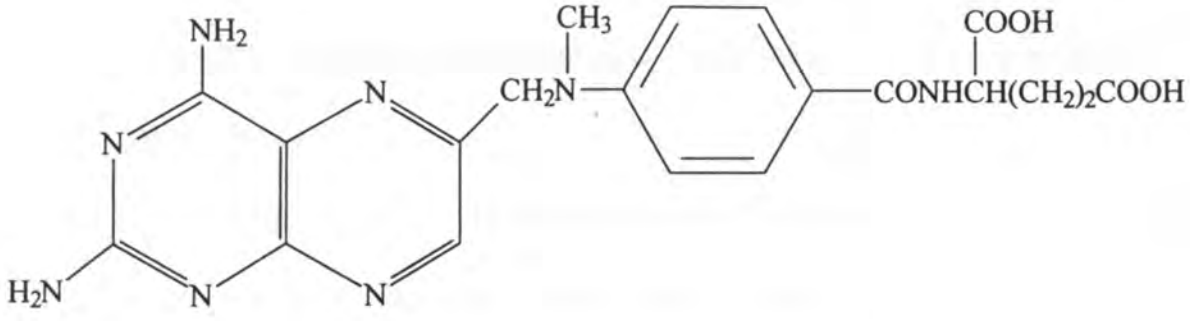
Sulfasalazine:

Sulfasalazine (Fig. 5) was developed over 50 years ago specifically for RA. The most common side effect is GI intolerance. Hematologic toxicity, including hemolytic anemia, folate deficiency, thrombocytopenia, leukopenia, and aplastic anemia are also seen. Rarely, reversible oligospermia, fever, rash, exfoliative dermatitis, and pneumonitis may develop (6).

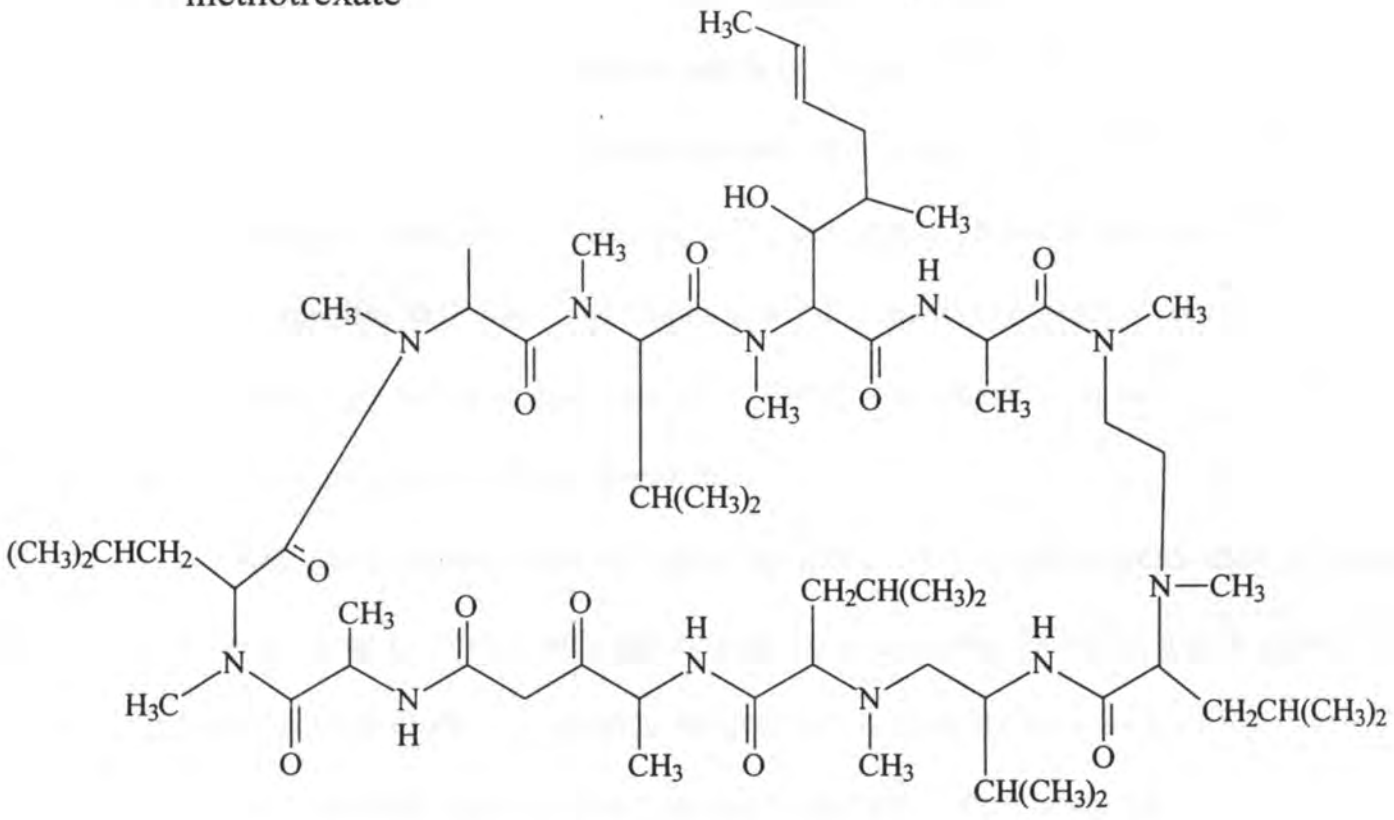
Cyclophosphamide:

Cyclophosphamide (Cytoxan®)(Fig. 5), an alkylating agent, has been shown to inhibit both humoral and cellular immunity and to suppress passive transfer of cellular immunity. The exact mechanism(s) of action is/are unknown. As an alkylating agent, cyclophosphamide interferes with cellular metabolism and exerts a cytotoxic effect independent of phases of the cell cycle. It appears not only to interfere with early events in the generation of the immune response by also to have a direct effect on B and T effector cells. Among various studies, the incidence of improvement of RA patients treated with cyclophosphamide ranged from 75 to 100%. Patients on this therapy appeared to show less radiological joint deterioration than patients treated with gold.

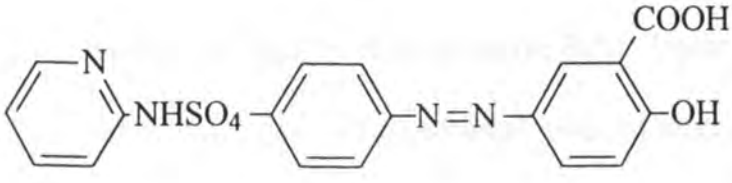
The actual mechanism of action of cyclophosphamide in RA is not understood. The drug appears to exert suppressive effects on some parameters of the immune response which may be of primary importance. There is a lack of correlation between therapeutic efficacy and immune status by any of the parameters measure to date. Studies have shown patients demonstrating decreased numbers of circulating lymphocytes. A decrease



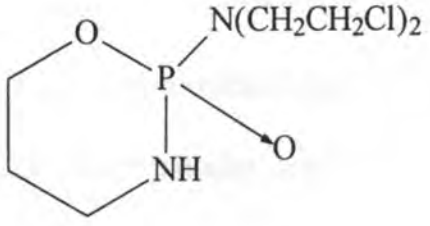
methotrexate



cyclosporine A



sulfasalazine



cyclophosphamide

Fig. 5

in both serum immunoglobulin and RF titers are also seen. Adverse side effects of cyclophosphamide include alopecia, hemorrhagic cystitis, azoospermia, and sustained lymphopenia (7).

A number of novel therapies are currently under development. A significant amount of emphasis is directed toward newer inhibitors of arachidonic acid metabolism, specifically inhibition of the 5-lipoxygenase pathway or drugs that inhibit the cyclooxygenase and 5-lipoxygenase pathway. These drugs are anticipated to have less GI toxicity than the traditional cyclooxygenase inhibitors. Compounds that affect specific cytokines, *e.g.* interleukin-1 are also being developed. Monoclonal antibodies directed against specific cell lines and compounds that inhibit proteolytic enzymes such as collagenase are being studied as well. Clinical trials have been initiated to test more selective immunosuppressive agents (6).

Anecdotal evidence has shown that insufflation of a modified preparation of cocaine has been found to provide substantial benefits in the treatment of RA and in slowing the progress of the disease. One such incident involves identical twins with RA. One was suffering from symptoms of the advanced stages of the disease while the other was symptom free. No traditional treatment seemed to be effective. The patient was deemed near terminal and was given cocaine to potentiate his pain medication. Within two weeks, he had no sign of active RA. Upon further questioning, the asymptomatic twin admitted to be a recreational user of cocaine (8). Treatment of other patients with similar disease profiles showed similar results (9).

These non-controlled studies led to the development of a topical preparation of

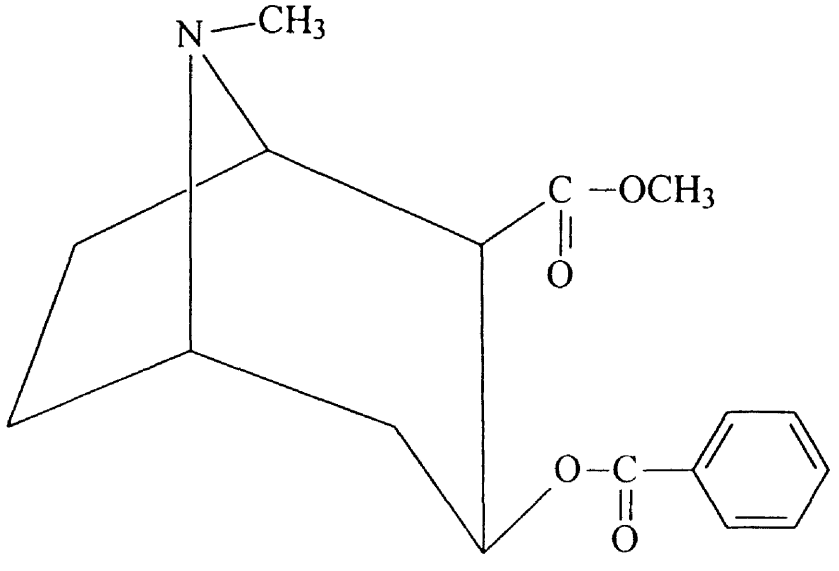
cocaine derivatives. It has been tested in Phase II clinical trials to determine efficacy in treating decreased range of motion due to painful shoulder, acute back sprain, or resulting from wearing a cast on a limb for an extended period of time. The results of these trials indicate that this preparation specifically improves restricted range of motion in painful shoulder and acute back sprain and is effective in improving range of motion following cast removal.

The results of these clinical trials corroborate the earlier anecdotal evidence. These compounds appear to be the origin of new drug entities that could alleviate the severest symptoms of RA and related conditions and even potentially arrest the progress of the diseases. Based on this possibility, the goal of this project is to utilize a logical structure activity relationship approach which leads to the development of the active agents. This approach includes developing proper synthetic procedures for and, structural analysis and biological screening of these compounds for potential activity. Cocaine is the starting material for the clinically evaluated topical preparation. This product is composed of a variety of cocaine derivatives. Consequently, the compounds proposed represent logical modifications of cocaine. A review of the chemistry, pharmacology and metabolism of cocaine follows.

COCAINE: STRUCTURE AND METABOLISM

Cocaine (I), (-)2(R)-carbomethoxy-3(S)-benzoxy-1(R)-tropane or 3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (11), a natural alkaloid, is obtained from the leaves of Erythroxylon coca, a plant species found in South

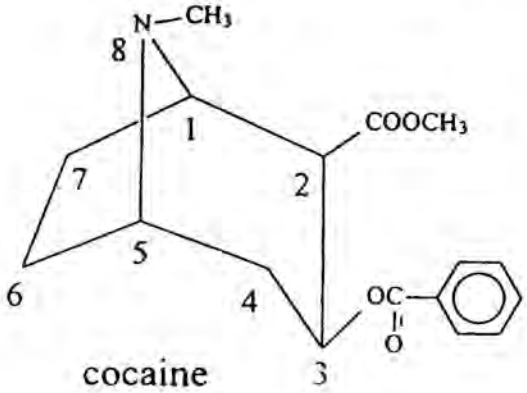
America. Cocaine (I) has two basic types of pharmacological properties. It can act as both a local anesthetic and CNS stimulant (10,11,12). Its conformation consists of the piperidine nucleus in the chair form with the carboxymethyl side chain on C-2 in the axial position and the benzoyloxy group on C-3 equatorial (Fig. 6). The molecule has four chiral centers on C-1, C-2, C-3, and C-5, giving rise to eight possible pairs of enantiomers. However, with C-1 and C-5 restricted to the *cis* position, and the fusion of the nitrogen bridge, only four pairs are actually possible. Three of the four pairs have been synthesized (10).



cocaine

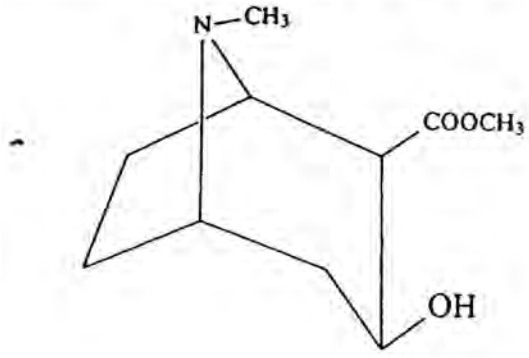
Fig. 6

Cocaine (I) is rapidly metabolized *via* hydrolysis by plasma esterases and liver enzymes or non-enzymatic hydrolysis to the water soluble products, methylecgonine (II) (ME) and benzoylecgonine (III) (BE) (11,12). The molecule is also N-demethylated to norcocaine (V) (NC), a pharmacologically active metabolite (10,11,13,14) (Fig. 7).



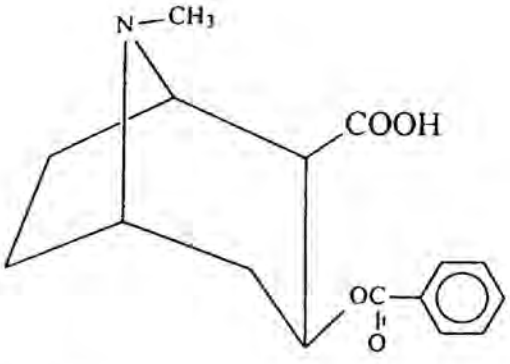
cocaine

(I)



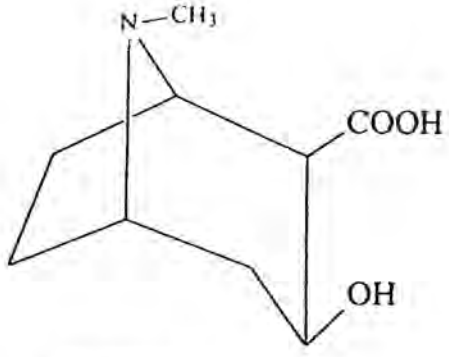
methylecgonine

(II)



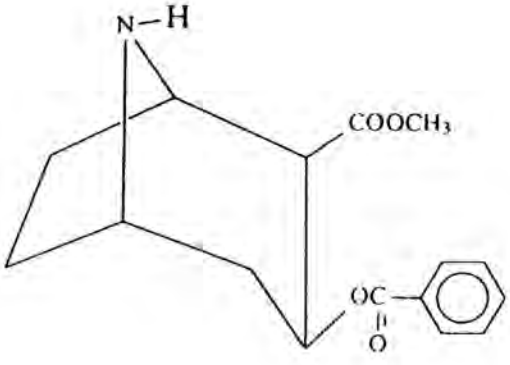
benzoylecgonine

(III)



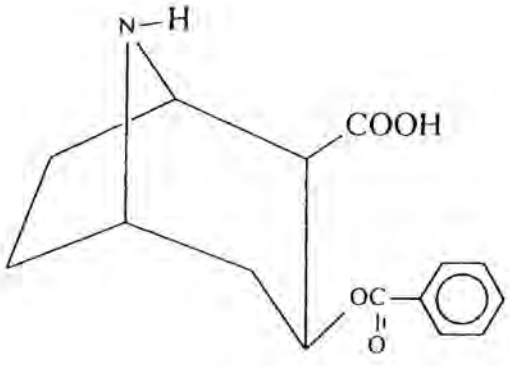
ecgonine

(IV)



norcocaine

(V)



norbenzoylecgonine

Fig. 7

PHARMACOLOGICAL PROPERTIES

Cocaine (I) possesses multiple actions on the central and peripheral nervous systems. Peripheral effects include potent local anesthetic properties as well as indirect catecholaminergic actions, and stimulatory effects in the central nervous system (16).

Local Anesthetic Actions

When applied to the skin or mucous membranes, cocaine (I) inhibits the conduction of sensory impulses within the neuronal membrane by reacting with the neuronal membrane to block ion channels (16-19). Membrane-bound calcium plays an important role in the initial phase of membrane depolarization necessary for the generation of nerve impulses. Cocaine (I) may inhibit this release of bound calcium or replace these ions at the site of action, thereby stabilizing the membrane to depolarization. This prohibits the production of the threshold potential required to permit the activation of the sodium ion channels which in turn prohibits the transmission of nerve impulses (17).

The other proposed route for cocaine's (I) actions as a local anesthetic involves disruptions in the neuronal membrane lipids, which increases the surface pressure of these membranes, causing closure of ion pores which restrict openings in the sodium ion channels, thus reducing ion permeability, resulting in the inhibition of nerve impulse transmission (16).

Stimulant Properties

The central and peripheral stimulant activities of cocaine (I) are due to the indirect catecholaminergic action that enhances the effects of nerve impulse transmission (16) .

Centrally, cocaine (I) acts on the nerve terminals for norepinephrine (NE)(12,16), dopamine (DA) (16), or serotonin (5-HT) (12). The peripheral actions are due to the potentiation of NE at the adrenergic terminals of the sympathetic branch of the autonomic nervous system (17).

Peripheral effects. Due to cocaine's (I) effects on NE, both the excitatory and inhibitory responses of sympathetically innervated organs are potentiated (17). The symptoms include tachycardia, vasoconstriction, hypertension, mydriasis, hyperglycemia, increased rate of glycogenolysis in the liver and muscles, liberation of fatty acids from adipose tissue, increased secretion of sweat glands, contraction of pilomotor muscles in the skin, stimulation of salivary glands, increased water and potassium secretion, and elevated body temperature (due to increased muscular activity and vasoconstriction) (12,17). Cocaine (I) blocks the reuptake of NE at the synaptic cleft and stimulates its release from presynaptic vesicles (17). Due to this, tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis, is activated. This neurotransmitter reuptake blockade increases *alpha*- and *beta*-adrenergic effects when the drug is used initially or acutely. Chronic use leads to a greater increase in *alpha* and *beta* receptor density with eventual sensitization (17). The overall effect is a decrease in NE levels at the peripheral adrenergic receptors.

Central effects. Both the euphoric and non-euphoric effects exhibited by cocaine (I) are due to its action on NE, DA, and 5-HT in specific sites in the brain. The non-euphoric effects are speculated to be caused by cocaine's (I) indirect action on NE and 5-HT. The symptomatic manifestations include anxiety, hypervigilance, suspiciousness,

and persecutory fears. The euphoric effects are exhibited primarily in the initial phase of use and are thought to be the result of cocaine's (I) indirect action on DA (12,16). They include mood elevation, a sense of increased self esteem, well-being, and mental and physical capacity. Appetite and need for sleep are decreased and sexual interest is heightened. Although the mechanism for the euphoria is not understood, it is speculated that cocaine blocks the reuptake of DA and also facilitates its release(12,17). These actions trigger the synthesis of DA and lead to an increase in the number of DA receptors and also an increase in receptor sensitivity. Ultimately, inhibition of DA vesicle binding occurs and causes the intracellular metabolism of more DA. Chronic use of cocaine (I) leads to decreased concentrations of DA in the brain(12,17). This depletion of DA can cause intense dysphoria, craving and symptoms of withdrawal. Neuroendrocrine dysfunction, such as hyperprolactinemia is also thought to result in this situation(10). Centrally mediated responses due to dopamninergic activity include increases in locomotor activity, body temperature, respiratory rate, alertness and loquaciousness; and decreases in appetite and fatigue (12,16). There is no doubt that the psychomotor stimulant effect is partly due to the ability of cocaine (I) to block reuptake of all central catecholamines (12,17).

Higher doses cause tremors and eventually tonic-clonic seizures which are possibility due to stimulation of the lower motor centers and an increase in the sensitivity of the cord reflexes. Continued use in elevated doses can be lethal due to depression of the medullary centers and respiratory failure (17).

Serotonin is a central neurotransmitter also capable of causing effects similar to

those of DA, as well as endocrine function. Cocaine (I) blocks 5-HT reuptake and facilitates its release, which will result in an overall decrease in the concentration of 5-HT, but just how cocaine (I) influences the functions of 5-HT is not clear (12,17).

STRUCTURE-ACTIVITY RELATIONSHIPS AND PHARMACOLOGICAL EFFECTS

Local Anesthetic Effects

The activities of cocaine (I) have been compared to benzoylecgonine (III) (BE), methylecgonine (II) (ME), ecgonine (IV) (E), and the stereoisomers (+)pseudococaine and (-) pseudococaine [the C-2 epimers], and (+/-)allococaine [the racemic C-3 epimer], as well as (+/-)allopseudococaine. All show varibility in both local anesthetic action (LAA) and influence on the sympathomimetics (17).

Methylecgonine (II) and ecgonine (IV) are both inactive in regard to LAA and have no effects on sodium channels(17). This may be due to the absence of the benzoyl group at C-3. Norcocaine (NC) (V) and (+)pseudococaine exhibit more local anesthetic activity than cocaine (I), while (+/-)allococaine and BE (III) are less potent than cocaine (I) (17). Removal of the ester linkage between the heterocyclic and aromatic rings on the molecule decreases the LAA (20,21).

(+/-)Pseudococaine has increased interaction with the sodium channels and increased LAA (21). Structural requirements for observable LAA include the benzoyloxy group equatorial at C-3, the ester group at C-2 either axial or equatorial, and the presence of a secondary or tertiary amine function. Also, the substituent on C-3 has more influence than the C-2 on sodium channel interaction (20).

Central Stimulatory Effects

The sympathomimetic activities of cocaine (I) and its cogeners do not correlate with the potential effects on the sodium channel (20). In studies, cocaine (I) and NC (V) are active both IV and intracisternally (17,19), while BE (III) and benzoylnorecgonine (VI) (BNE) are active after intracisternal administration only. Ecgonine (IV) and ME (II) are inactive *via* both routes. The effects of BE (III) and BNE (VI) by intracisternal injection are dose dependent and differ in nature from those seen after injection of cocaine (I) and NC (V) (17).

Structurally, the diester is required for both LAA and absorption across the blood brain barrier. The metabolites with the carboxyl groups at C-2 [BE (III), BNE (VI)] exhibit stimulatory activity once they are in the CNS. The secondary amine such as NC (V) has greater stimulatory activity than the tertiary amine(17).

Calcium Binding Effects

Cocaine (I) and its metabolites have been studied in an attempt to parallel the observed CNS effects with the calcium binding activity of the compounds. Calcium is known to participate in several neuronal functions at the membrane level, such as neuronal excitation. Increased calcium ion concentration greatly increases the threshold of nerve stimulation while decreased calcium increases nerve excitability(19). Due to the complexation of calcium by cocaine (I) or its metabolites and subsequent mobilization of neuronal-membrane bound calcium ions which decreases the availability of the ion to sites controlling transmembrane permeability to sodium ions, the excitatory effects of cocaine and its metabolites may be greatly influenced(19).

Neuronal Uptake Activity

The structure activity relationship of cocaine (I) and its cogeners has been evaluated in light of the structural requirements for interaction with DA and 5-HT neuronal uptake sites in mouse brain (20). Removing the ester linkage between the phenyl and tropane ring did not decrease the affinity for either cocaine (I) binding sites or the monoamine uptake sites. Conformation has significant effects, as pseudococaine (C-2 epimer) and allococaine (C-3 epimer) show decreased potency with allococaine being the least potent of the two, suggesting that the position of the substituent at C-2 is more important in DA and 5-HT uptake. Removing the C-2 substituent decreases this activity greatly, although less than removal of the methyl ester at C-2 [BE (III), E (IV)] which inactivates the molecule. This may be due to the reduced lipophilicity and therefore decreased affinity. Differences in activity are also seen in the *dextro* and *levo* isomers of some of the modified compounds, revealing that stereoselectivity is a factor for both sites(20) .

PROPERTIES PERTAINING TO ABUSE POTENTIAL

The most relevant effects of cocaine (I) pertaining to drug abuse include its ability to produce euphoria and its reinforcing properties. In addition, cocaine (I) has properties similar to other drugs of abuse such as withdrawal and craving (22).

Of the sites of action of cocaine (I) in the CNS, it is the dopaminergic pathway that seems to be responsible for cocaine's (I) reinforcing properties. The theory for this is the "Dopamine Hypothesis" (25). This hypothesis puts forth the premise that cocaine

(I) binds at the DAT and inhibits neurotransmission reuptake in the mesolimbocortical pathways and thereby causes reinforcement. Ligand binding studies present evidence that binding at the DAT is inhibited by cocaine (I) and by related compounds at potencies of the same compounds in behavioral studies of drug reinforcement (26). Since ligand binding with other receptors do not show such a correlation, cocaine's (I) reinforcing properties can be specifically related to binding at the DAT.

Pharmacological studies of drug self-administration support the hypothesis (28-30), although there is the possibility that cocaine (I) has activity other than the inhibition of DA uptake. Pharmacokinetic effects probably play a significant role since drugs entering the brain more rapidly are more likely to induce dependence (31) and cocaine is known to cross the blood brain barrier and quickly occupy receptors(32,33).

Specific evidence supporting the hypothesis include the following(24):

1. The potency of various cocaine-like compounds in maitaining self-administration can be predicted by each compounds' affinity for the DAT but not predicted by its affinity for NE or 5-HT transporters.

2. A lesion of the dopaminergic innervation of the nucleus accumbens disrupts self-administration of cocaine (I), but similar lesions in noradrenergic system or DA terminal in the striatum have no effect.

3. Cocaine (I) craving in humans is decreased by indirect agonists of DA (34,35).

4. The increase in extracellular DA levels in the brain following cocaine (I) administration has a time course similar to the euphoria reported by the subjects taking

cocaine (I).

There is some controversy as to the type of binding that occurs between cocaine and its binding site on the DAT. Evidence supports both competitive and allosteric binding. Several compounds including cocaine (I) have been shown to competitively inhibit DA uptake in striatal tissue (37,46). Other study results support the hypothesis of allosterism; that cocaine (I) inhibits the DAT by binding to a site other that the DA recognition site on the transporter(38,39,40). Also, the fact that DA is more potent that cocaine (I) in inhibiting [^{3}H]DA accumulation *in vitro,* but is much less potent than cocaine in displacing specifically bound [^{3}H]-(R)-cocaine is consistent with the ideas of different recognition sites (41, 42).

Two early studies reported evidence supporting a single [^{3}H]-R-cocaine binding site to mammalian brain (39,40). Present evidence has demonstrated that there are at least two binding sites for (R)-cocaine associated with the DAT, one with high affinity and one with low affinity. The two sites have kd=0.1-210 nM and 2.57 nM to 26.4 μM, depending on the radioligand, tissue source, species and buffer used. One key question pertains to whether the two cocaine (I) binding sites are related to two distinct proteins, distinct sites on the same protein or to two different affinity states of one protein (42).

Since clinical effects of cocaine (I) are seen at plasma concentrations of less than 1.0 μM (40), the high-affinity site may be the most physiologically significant receptor site, although quite a few of the low-affinity sites will be occupied at this concentration as well. Whether this site alone is relevant to cocaine's (I) abuse potential or whether both sites play a role in the reinforcing properties of cocaine (I) is unknown.

An understanding of the SAR of cocaine (I) is necessary to gain an insight into the interactions of cocaine (I) with the DAT that seem to be implicated in the CNS effects of the compound. A comparison of the effects of structural variation on the IC50 values for the inhibition of binding and DA uptake at the DAT is a means by which this understanding may be gained. The results of several SAR studies have been reported (2,17-19). The conclusions include the following requirements:

a) (R)-configuration of cocaine (I) structure (2,17);

b) a *beta* substituent (17,18) at C-2, preferably a carbomethoxy group (17);

c) a benzene ring at C-3, an ester link in this position is not required (17).

Substitutions at C-2 (19), C-3 (17), N-8, the effects of stereochemistry (2), and position of the ring nitrogen (18) have been further elucidated.

An analysis of the effect of a single structural parameter on the activity of cocaine-like compounds at the DAT was done and the following observations were made (27):

1) The largest factor in activity is configuration. Inversion of configuration decreases activity by a factor of up to 1000. This effect is not carried over into cocaine's (I) less active isomers. The activities of (S)-pseudococaine, (S)-allococaine and (S)-allopseudococaine are only 1.2-7.4 times smaller than those of the R analogues.

2) The second largest influence on activity is substitution at C-2. Replacement of the carbomethoxy group by H, COOH, or by an N-methylcarboxamido group reduces the activity by 25-2000 fold. Replacement by acetoxymethyl or hydroxymethyl groups decreases the activity by factors ranging from two to five. It appears that a variety of ester groups can be accomodated without substantially changing the compound's activity.

These initial results suggested the presence of at least one and possibly two H-bond acceptor sites localized in the vicinity of the two oxygens in the 2-*beta*-carbomethoxy group (Fig. 27). However, this premise has been challenged by Kozikowski and co-workers(44,45), who in recent years have synthesized and evaluated various cocaine analogs modified at C-2. Specifically, the carboxymethyl group was replaced with various moieties that are incapable of strong hydrogen-bonding. These groups include substituted isoxazoles and isoxazolines (44), and vinylic analogues of cocaine (45), as well as similar structural analogues of the 3-*beta-para*-chlorophenyl WIN compound (44,45). Analogues of the WIN-type compound were also synthesized in which the C-2 vinylic group was hydrogenated to remove the *pi* electrons (46).

These were then evaluated for their ability to block [^{3}H]DA reuptake and [^{3}H]mazindol binding. The results of each of the studies indicated a lack of specific hydrogen-bond donor groups within the region of the cocaine (I) recognition site which surrounds the C-2 ester group and the probability of a different binding modality being available for the alkyl or alkenyl analogues versus their C-2 ester counterparts (46).

3)The stereochemistry at the C-2 center has a significant impact on activity. Specifically, epimerization from the *beta* to the *alpha* configuration results in a decrease in activity by factors ranging from 30-200. This effect is not seen to this extent in compounds with a C-3-*beta*-hydroxyl or C-3-*alpha*-substituents (24).

4) Substitution at the nitrogen can have a fairly significant effect on the activity as well. Replacement of the N-methyl with an allyl or benzyl reduced activity by a factor of seven or less. Replacement by an acetyl to form an amide or addition of a methyl

group to create quaternary salt decreased activity by factors of 33 and 11, respectively.

The above initial evidence suggested that a reduction in the electron density of the nitrogen was detrimental to activity. However, in a recent study, N-sulfonylated analogs were synthesized, evaluated, and found to have activity comparable to or more potent than cocaine (I) in mazindol-binding and DA uptake experiments(48). Specifically, a trifluoromethanesulfonyl group ,one of the strongest electron withdrawing groups available (47), was substituted for the N-methyl thereby making the nitrogen electrons unavailable for protonation. This analogue had a potency equal to cocaine (I) while a sulfonylisocyanate derivative was two-fold more potent (48).

The suggestion that a basic nitrogen is necessary for binding to the cocaine (I) recognition site would seem to be logical since at physiological pH, the nitrogen is protonated and would be attracted by the negatively charged carboxylate (51) at the aspartate-79 residue of the transporter molecule. However, the data above would tend to indicate that a neutral nitrogen bridge can provide high-affinity ligands and that a basic nitrogen is not an absolute requirement for binding (48). Ionic bond type drug-receptor interactions have a bond strength estimated to be eight to ten Kcal/mol (50), and since these sulfonamide derivatives cannot participate in this type interaction, they would be expected to have a much lower potency in comparison to cocaine (I). The binding of these analogues is more likely to be due to hydrogen-bonding or weak polar interactions. This is supported by the fact that those compounds exhibiting the possibility of additional interactions of these types show even higher affinity. The residue of the transporter that is the recognition site of the cocaine nitrogen may be one which can be either a

hydrogen-bond donor or acceptor such as an OH group on a tyrosine (48). This hypothesis would at least partially explain the report that the N-nitro derivative was found to be 13 times more potent that the N-nitroso (49), the former having one additional H-bond acceptor site than the latter. Since the Asp-79 has been found to be necessary for DA uptake, it may be possible that the recognition site for cocaine and DA may overlap, but are not comprised of the exact amino acids (48).

SPECIFIC AIMS

The overall goals of this project are the following:

1. Synthesis and structural elucidation of a series of cocaine analogs by alkylation and acylation of the amine nitrogen on position eight of the tropane ring. The specific compounds to be developed are the ethyl, *n*-propyl, isopropyl, *n*-butyl, benzyl, acetyl, propionyl, butyryl, and benzoyl derivatives.

2. Biological evaluation of these cocaine analogs on ligand binding and dopamine uptake.

3. Synthesis and structural elucidation of a series of 3-*beta* esters of ecgonine and their amide isosteres.

4. Synthesis and structural elucidation of a small series of compounds in which the 3-carboxylic acid moiety of benzoylecgoinine is reduced to a primary alcohol and esterified with various NSAIDs. These compounds are potential prodrugs for both the esterified NSAID and for benzoylecgonine.

EXPERIMENTAL METHODS AND RESULTS

SUPPLIES AND EQUIPMENT

Chemicals

Sodium Chloride 99+% ACS, Sodium Carbonate granular 99.5+% ACS, Sodium Hydroxide pellets 97+% ACS, Potassium Hydroxide pellets 85+% ACS, Sodium Sulfate 99% ACS, Methyltrifluoromethane-sulfonate 99+%, 1-Chloroethyl Chloroformate, 98%, Nitromethane 95+%, Propionic Acid 99+%, 1,1'-Carbonyldiimidazole, Acetylsalicyloyl Chloride 95%, Acetylsalicylic Acid +99%, Butyric Acid 99+%, Benzonitrile, Calcium Hydride 90-95%, Acetone 99.5%, Acetyl Chloride 99+%, Benzaldehyde 99+%,Sodium Cyanoborohydride 95%, Butyryl Chloride, Borane-Tetrahydrofuran Complex, Aldrich Chemical Company Inc., Milwaukee, WI, Potassium Permanganate ACS, Butyric Anhydride 99%,Charcoal Activated, Salicylic Acid, Tetramethylsilane 99.9%, Acetic Anhydride, Indomethacin, Ibuprofen, Postassium Carbonate Anhydrous ACS, Propionic Anhydride, Sigma Chemical Company, St. Louis, MO, Ethyl Acetate GC, HPLC, Acetone, Tech Grade, Baxter Healthcare Corporated, Muskegon, MI, Ethyl Ether Anhydrous, AR, Sodium Bicarbonate, Chloroform AR, Methyl Alcohol Chromar HPLC, Methylecgonine HCl, Cocaine HCL, Mallinckrodt, Paris, KY, Hexane 97% *n*-hexane (HPLC), Ammonium Hydroxide 30%, Instra-analyzed reagent, Trace Metal Analysis, J.T. Baker,

Phillipsburg, NJ, Acetonitrile UV, HPLC, GC, Baxter Burdick and Jackson, McGraw Park, IL

Sulfuric Acid ACS, Hydrochloric Acid ACS, Acetic Acid Glacial Acetic Acid,Toluene ACS, 1,4-Dioxone ACS, Darco 6-60 Activated Carbon, Acetic Anhydride ACE, Fisher Scientific, Fairlawn, NJ

Glassware

All reactions unless otherwise noted were carried out in Kimax glassware. The types and volumes are listed in each reaction procedure.

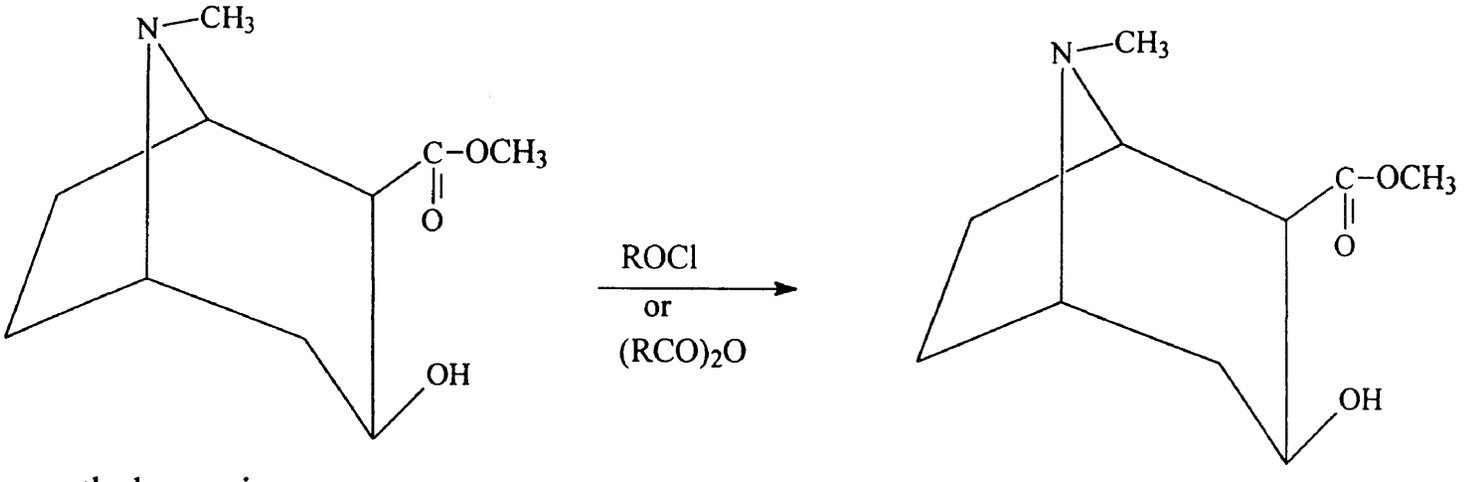
Equipment

A Yamato HiTech Rotary Evaporator Model RE-51, Yamato water bath Model HT-51, and Gast Vacuum Pump Model No. 0523-U4B-6180DX were used for reduced pressure evaporations. Melting points were obtained on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. Corning Hot Plate/Stirrers were used to maintain constant stirring and/or temperature during the reactions. Solid probe GCMS analyses were performed on a Finnigan Model 9610 gas chromatograph-4000 Series mass spectrometer. Other GCMS analyses were conducted on a Finnigan MAT5100 series gas chromatograph-mass spectrometer. FT-IR analyses were performed on a Mattson Polaris IR fitted with an HP 7470A plotter. Samples were dissolved in chloroform, placed on the NaCl plates, and allowed to evaporate. Elemental analyses were performed by

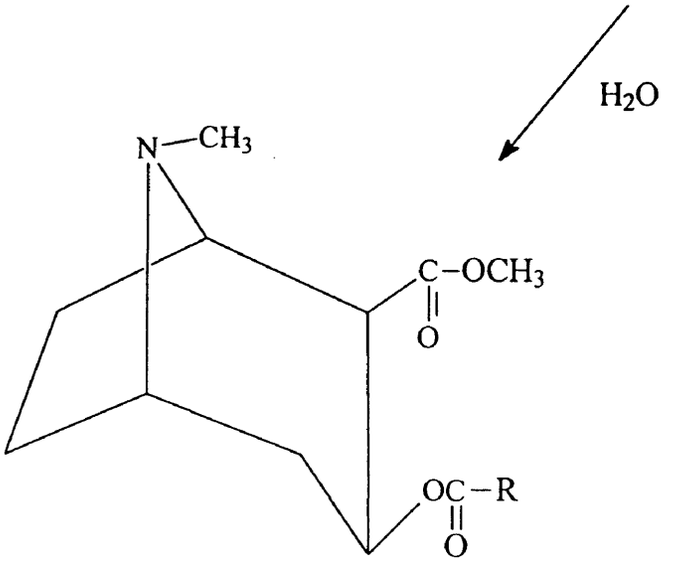
Atlantic Microlab P.O. Box 2288, Norcross, GA, 30091, for C, H, and N composition. Nuclear Magnetic Resonance Analyses were performed on either a Varian Gemini 300 (300 MHz) or a Varian VXR-400 (400 MHz) NMR with conventional quadrature. Chemical shifts are reported in δ ppm downfield from and internal tetramethylsilane (TMS) standard or in relation to residual $CHCl_3$.

SYNTHETIC PROCEDURES

Attempted Esterification of Methylecgonine (II) Utilizing Anhydrides or Acid Chlorides



methylecgonine



ecgonine ester

SAR Series
R=methyl
ethyl
propyl
butyl
phenyl

Other compounds of interest
R=2-aceyloxyphenyl
2-hydroxyphenyl

Scheme 1

Anhydrides

Procedure 1. Methylecgonine (II)(ME) (1.00 g, 5.0 mmol) was suspended in a mixture of acetonitrile (ACN) (40.0 mL) and pyridine (1.0 mL), contained in a 100 mL round-bottomed flask fitted with a relux condenser column and a magnetic stirrer. The mixture was cooled to 0 °C and the appropriate anhydride (7.0 mmol) was added dropwise over 6 to 10 min. The reaction mixture was refluxed for 48 hr, and allowed to come to room temperature. Water (60 mL) was added and the volume of solution was evaporated to 10% of the original volume under reduced pressure to remove the pyridine. A solution of sodium bicarbonate (10%) was added to the resulting residue with swirling until the mixture was basic to litmus. This alkaline mixture was extracted five times with (5×10 mL) of ether. The organic layers were combined and evaporated to 25% of the original volume under reduced pressure and then allowed to evaporate completely in the hood overnight. A total of five attempts were made using both butyric anhydride and acetic anhydride.

Procedure 2. Nitromethane (CH_3NO_2) (10 mL) and triethylamine (0.4 mL), ME (II)(100 mg, 0.5 mmol), was dissolved in a 50 mL Erlenmeyer flask fitted with a magnetic stirrer. The appropriate anhydride (1.2 mmol) was added dropwise over 6 to 10 min with continuous stirring. The reaction mixture was allowed to remain at room temperature under nitrogen for 24 hr, water (15 mL) was added and the volume reduced to 10% of the original by under reduced pressure. Sodium bicarbonate (10%) was added to the remaining solution until it became basic to litmus and then this aqueous mixture

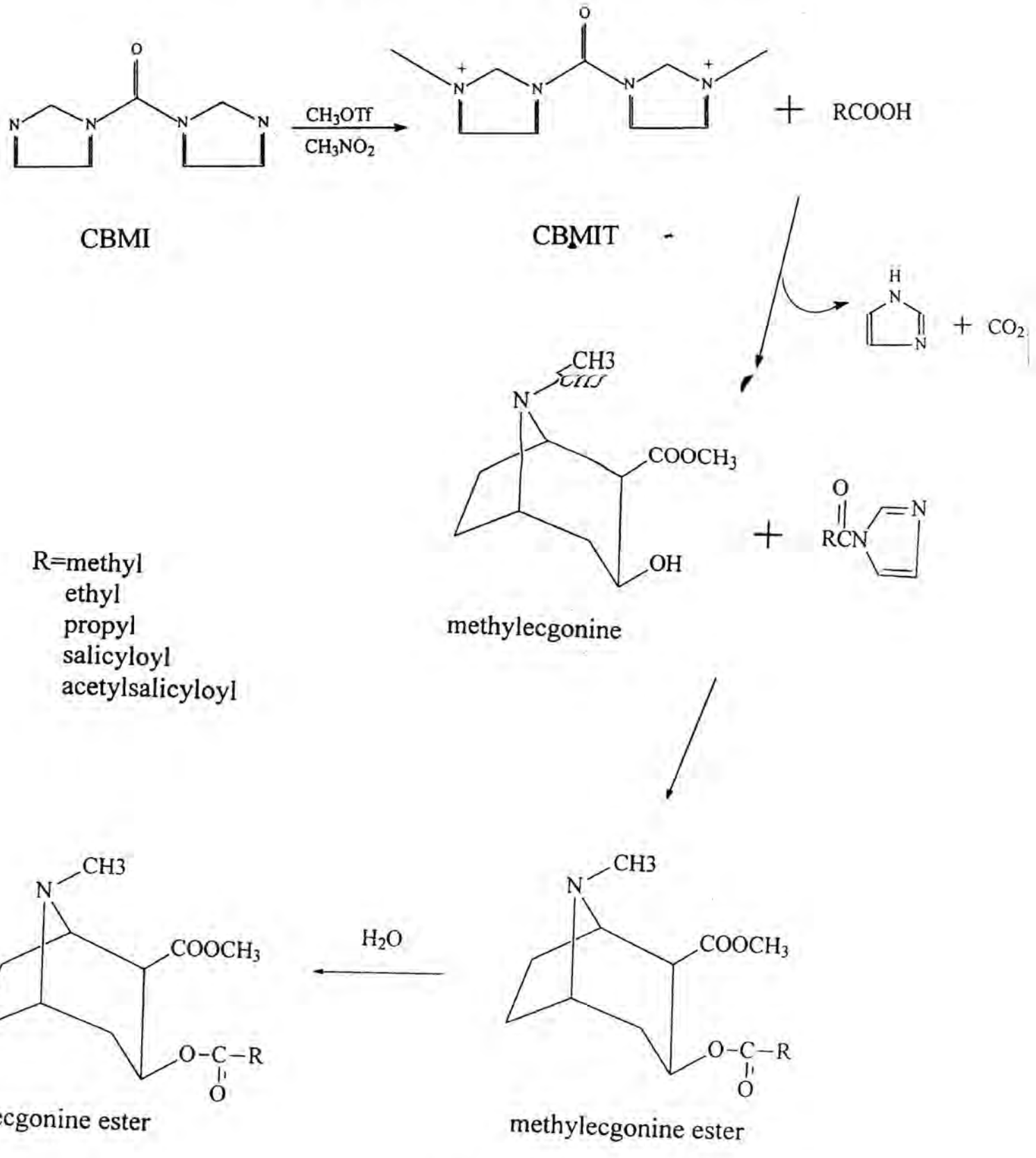
was extracted three times with (3×5 mL) of ether. The organic extracts were combined and washed with brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate allowed to evaporate from an open container in the hood. Two reaction attempts were made with both acetic anhydride and butyric anhydride.

Acid Chlorides

Methylecgonine HCl (490 mg, 2.09 mmol) was added to 40 mL ethyl acetate contained in a 100 mL round-bottomed flask fitted with a reflux condenser and magnetic stirrer. Pyridine (4.0 mL) was then added. The appropriate acid chloride (2.6 mmol) was dissolved in 4 mL of ethyl acetate and added dropwise over 10 min with continuous stirring. The reaction mixture was heated to 70 °C for 24 hr, allowed to come to room temperature and filtered. The solvent was removed under reduced pressure and approximately 20 mL ether added to the residue. The organic layer was extracted three times with (3×20 mL) of distilled water. The ether layer was then allowed to evaporate from an open container in the hood. A total of seven reactions were attempted utilizing acetylsalicyloyl chloride (3), salicyloyl chloride (2), and butyryl chloride (2).

For each of the above procedures, specific products present in the reaction mixture were identified *via* GC/MS. However, none could be isolated in quantities sufficient for analytical characterization or yield determination.

Attempted Esterification of Methylecgonine (II) Utilizing Carbonyldiimidazole



Scheme 2

Methyl trifluromethanesulfonate (CH_3OTf) (1.13 mL, 10 mmol) was added dropwise to a cooled solution (ice water bath) of carbonyldiimidazole (CBMI) (810 mg, 5 mmol) in 10 mL of redistilled nitromethane (CH_3NO_2) contained in a 50 mL Erlenmeyer flask fitted with a magnetic stirrer. This solution was added dropwise over 6 to 10 min to a suspension of the appropriate acid (5 mmol) in 10 mL of CH_3NO_2 contained in a 50 mL round bottomed flask fitted with a reflux condenser column and a magnetic stirrer. After 5 min, ME (II)(400 mg, 2 mmol) was added. The reaction mixture was refluxed under nitrogen for 80 hr, and allowed to come to room temperature. The reaction was quenched with 5 mL water and extracted into 15 mL of ether. The ether extract was washed twice with (2×20 mL) of saturated $NaHCO_3$ and twice with (2×20 mL) of brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and each solution of crude esters were allowed to evaporate to dryness under the hood.

Each product was analyzed using GC/MS. The GC/MS spectra indicated the presence of the acetate (IIa), propionate (IIc) and butyrate (IId) esters. Not enough of the acetate (IIa) nor butyrate (IId) esters could be isolated for analysis, and these crude mixtures were used in an attempt to make the 2-carboxylic acid derivatives. Using preparative thin layer chromatography (PTLC), only the propionic acid ester (IIc) of ME was isolated and only in a quantity sufficient to perform GC/MS analysis. In each reaction, the remaining material was unreacted ME (II).

ANALYTICAL:

[1R-(*exo,exo*)]-3-propionyloxy-8-ethyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (IIc): GC/MS m/z 255 (M+,12.33), 198 (6.85), 182 (60.27), 94 (41.10), 82 (100), 57 (17.81).

In the reactions using salicylic and acetylsalicylic acids, the GC/MS revealed a complex mixture of products including minute amounts of each of the desired esters. The GC/MS analysis indicated that none of the mixtures contained enough of the desired products to allow isolation and structural characterization.

Attempted Hydrolysis of the 2-Methyl Ester Products to Yield the 2-Carboxy-3β-Esters (See Scheme 2)

The crude ester mixtures produced in the above synthetic reactions were suspended in water in a 100 mL round-bottomed flask fitted with a reflux condenser and magnetic stirrer. The reaction was refluxed for 4 to 5 hr and allowed to come to room temperature. The aqueous solution was extracted 3 times with (3×30 mL) portions of ether, and the aqueous portion was evaporated to near dryness under reduced pressure. The flask was stoppered and placed in the refrigerator overnight. No crystals formed. The solution was evaporated to dryness under reduced pressure. Absolute alcohol was added and the solvent was removed under reduced pressure. For each reaction, GC/MS indicated the presence of ecgonine (IV) and ecgonidine as well as small amounts of the desired free acid. Only the butyric acid ester (IVd) was isolated in any quantity (11.0 mg, 5.0% crude yield).

ANALYTICAL:

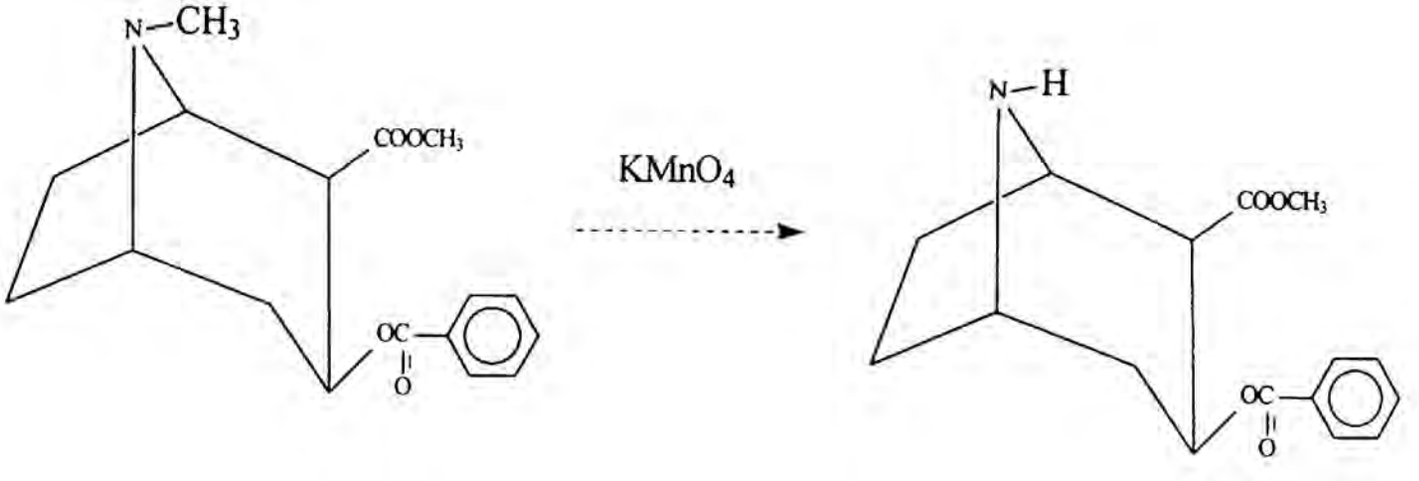
[1R-(*exo,exo*)]3-burtyryloxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid

(IVd): GC/MS m/z 254 (M+,6.80), 168 (74.8), 124 (100), 108 (5.44), 87 (25.85)

N-MODIFIED COMPOUNDS

Procedures for N-Demethylation

Findley's Method



cocaine norcocaine

Scheme 3

Benzoylecgonine (III)(BE) (1.53 g, 5.3 mmol) was dissolved in 140 mL water and 69 mL acetonitrile contained in a 500 mL round-bottomed three neck flask fitted with a pH meter and magnetic stirrer. Potassium permanganate (1.35 g, 8.54 mmol) dissolved in 52 mL water was added dropwise over 10 hr. The pH was kept slightly acidic by addition of a solution of 40% acetic acid (HOAc). The reaction was allowed to stir overnight at room temperature. Methanol (MEOH) (10 mL) was added and the solution stirred for 1 hr. Activated charcoal and celite were added and the mixture stirred for 30 min and then vacuum filtered. This procedure was repeated until the filtrate was colorless. The filtrate was then treated with HCl (56 mEq) and evaporated to dryness under reduced pressure. The residue was stored *in vacuo* over KOH overnight. The residue was dissolved in absolute alcohol, heated to boiling and filtered. The filtrate was concentrated by evaporation under reduced pressure and placed in the refrigerator. No precipitate formed. The remaining solvent was evaporated under reduced pressure and an ^1H NMR performed. The ^1H NMR spectrum indicated that the material isolated was BE (III).

Meltzer's Method Modified (See Scheme 4)

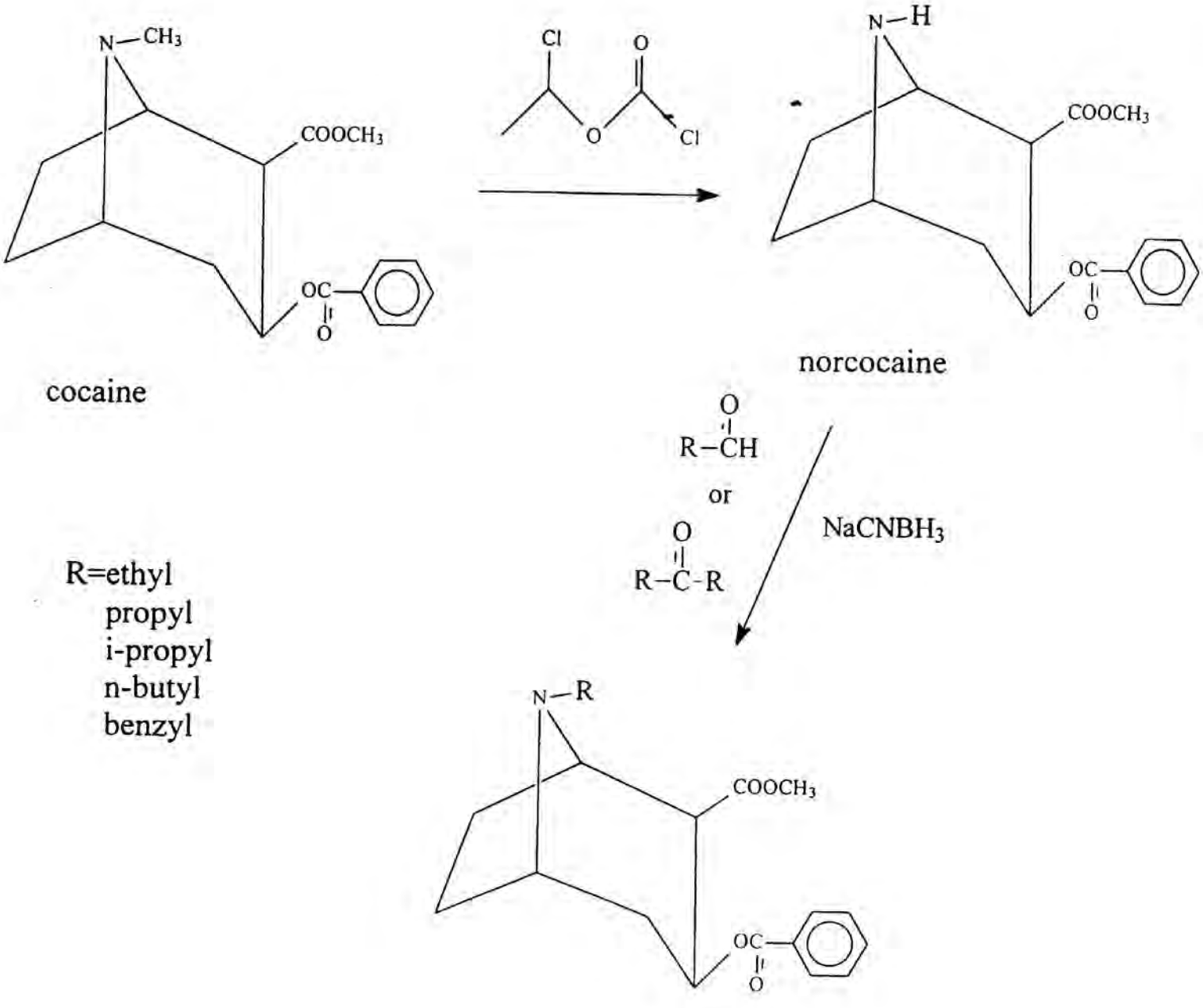
Cocaine (I)(5.00 g, 16 mmol) suspended in α-chlorethyl chloroformate (ACE-Cl) (15 mL) was placed in a 50 mL round-bottomed flask fitted with a reflux condenser and magnetic stirrer. The flask was placed in an oil bath, heated to 110-115°C, and refluxed under N_2 for 24 hr. The reaction was allowed to come to room temperature and the solution was evaporated to dryness under reduced pressure. The residue was dissolved

in a minimum of CH_2Cl_2, and cold 2N HCl added until the reaction mixture was acidic to litmus. This acidic mixture was extracted twice with (2×15 mL) of water to remove the unreacted cocaine HCl. The organic component was evaporated to dryness under reduced pressure, a minimum of MEOH added and heated to 60°C overnight. The reaction was then allowed to come to room temperature and was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum of CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The product was found to be analytically pure norcocaine (V)(NC) by GC/MS. The product was an amber oil weighing 4.60 g (96% yield)

ANALYTICAL:

Norcocaine (V): oil 96% yield; GC/MS m/z 289 (M+,9.59), 184 (28.77), 168 (21.90), 152 (2.70), 105 (100), 77(78.08), 59 (12.33) ; FT-IR cm^{-1} 2934, 2952 (ring CH_2), 1733,1717 (carboxyls), 1456 (aromatic), 1274 (2° amine), 715 (NH 2° αC); ^1H NMR (300 MHz, $CDCl_3$) δ 2.5 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.4 (m,2H,C_5-H,C_1-H), 3.6 (s,3H,OCH_3), 5.0 (m,1H,C_3-H), 7.2-8.0 (m,5H,Ar-H).

Procedure for N-Alkylation



Scheme 4

Norcocaine (V)(NC) (2.01 g, 6.9 mmol) was dissolved in MEOH (40 mL) in a 100 mL flask fitted with a magnetic stirrer and rubber septum. The appropriate aldehyde or ketone (8.1 mmol) was added with a syringe under N_2 over 6 to 10 min. Sodium cyanoborohydride ($NaCNBH_3$) (0.240 g, 3.82 mmol) was dissolved in a minimum of MEOH and added with a syringe to the reaction mixture. The reaction was allowed to stir at room temperature for 18 hr. The solution was made acidic to litmus with 1N HCl and extracted 3 times with (3×40 mL) ether and the ether extracts were discarded. The aqueous layer was made basic to litmus using a solution of 5% $NaHCO_3$ and extracted 3 times with (3×40 mL) ether. The organic extracts from the second extraction process were combined and dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure. Each tertiary amine product was recrystallized from hexane.

Acetaldehyde, propanaldehyde, butyraldehyde and benzaldehyde were used in the above procedure. The ketone used was acetone.

ANALYTICAL:

[1R-(*exo,exo*)]-3-benzoyloxy-8-ethyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Va): MP 135 °C, 28% yield (hexane); GC/MS m/z 289 (5.56), 258 (2.42), 184 (20.92), 152 (2.07), 108 (9.53), 105 (100), 77 (76.67); FT-IR cm^{-1} 2954 (ring CH_2), 1740, 1734 (carbonyl), 1601 (aromatics), 1457 (alkyl), 1437 (CH_2-N), 1234 ($COOCH_3$), 668 (aromatics); ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (t,3H,N-CH_2-**CH_3**), 2.3 (q,2H,N-CH_2), 2.6 (m,1H,C_4-H), 3.1 (m,1H,C_2-H), 3.3 (m,2H,C_5-H,C_1-H), 3.5

(s,3H,OCH_3), 5.2 (m,1H,C_3-H), 7.1-8.1 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{18}H_{23}NO_4$: C, 66.25; H, 6.65; N, 4.81. Found: C,66.25; H,6.68; N, 4.79.

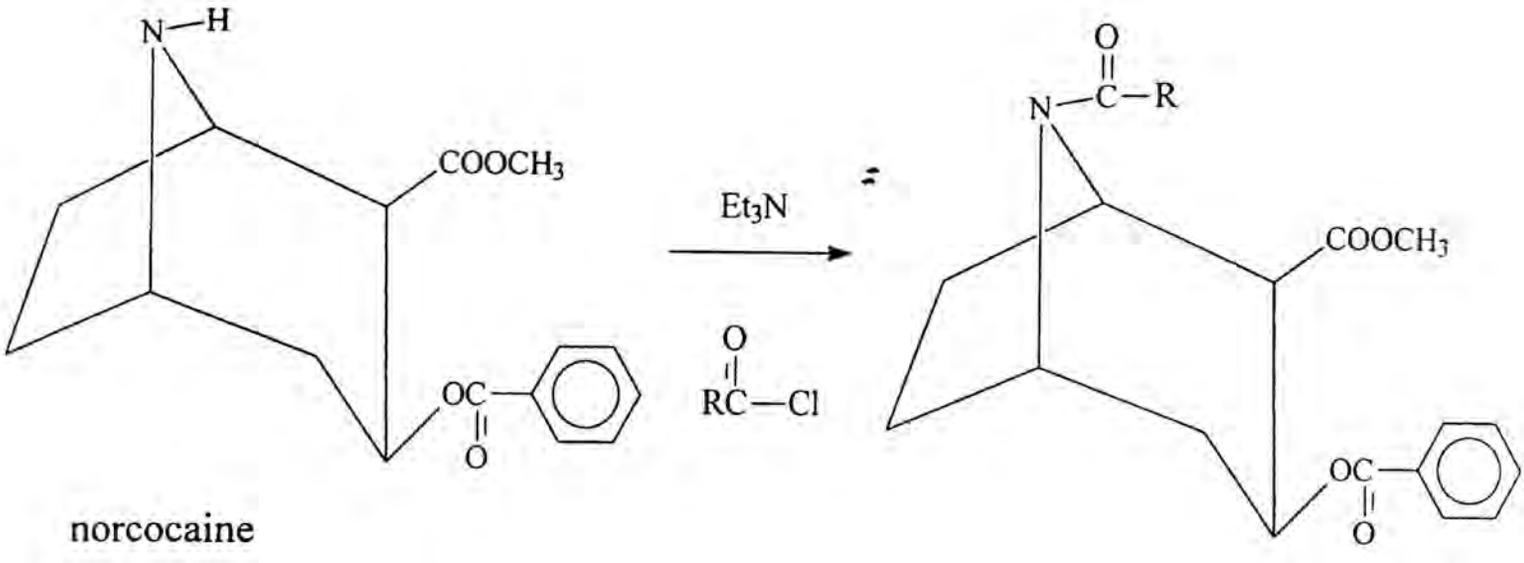
[1R-(*exo,exo*)]-3-benzoyloxy-8-propyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Vb): MP 135 °C, 33% yield (hexane); GC/MS m/z 288 (4.74), 184 (21.00), 168 (11.90), 136 (6.21), 122 (1.25), 105 (100), 77 (79.96), 59 (21.73); FT-IR cm^{-1} 2955, 2921 (ring CH_2), 1734, 1718 (carbonyl), 1616,1601(aromatic), 1457(alkyl), 1447(OCH_3), 1437(N-CH_2); ^1H NMR (300 MHz, $CDCl_3$) δ 1.0 (t,3H,N-CH_2-CH_2-CH_3), 1.8 (m,2H,-CH_2), 2.3 (q,2H,N-CH_2), 2.6 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.3 (m,2H,C_5-H,C_1-H), 3.5 (s,3H,OCH_3) , 5.1 (m,1H,C_3-H), 7.1-8.1 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{19}H_{25}NO_4$: C, 66.88; H, 7.53; N,4.21. Found: C,68.86; H,7.50; N, 4.20.

[1R-(*exo,exo*)]-3-benzoyloxy-8-isopropyl-8-azabicyclo[3.2.1]octane-2-carboxylicacid, methyl ester (Vc): MP 130 °C, 31% yield (hexane); GC/MS m/z 331 (M+, 7.64), 300 (4.33), 226 (9.73), 210, (49.34), 122 (23.51), 105 (86.75), 77 (100), 59 (12.05); FT-IR cm^{-1} 2955,2921 (ring CH_2), 1734, 1718 (carbonyl), 1616, 1601 (aromatic), 1457 (alkyl), 1447 (OCH_3), 1437 (CH_2-N); ^1H NMR (300 MHz,$CDCl_3$) δ 0.8-1.0 (s,6H,N-CH_2-($\mathbf{CH_3}$)$_2$), 2.2 (m,1H,N-CH), 2.6 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.3 (m,2H,C_5-H,C_1-H), 3.6 (s,3H,OCH_3), 5.2 (m,1H,C_3-H), 7.4-8.0 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{19}H_{25}NO_4$: C, 68.88; H, 7.53; N, 4.21. Found: C,68.85; H, 7.50; N, 4.20.

[1R-(*exo,exo*)-3-benzoyloxy-8-(n-butyl)-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Vd): oil, 37% yield (hexane); GC/MS m/z 345 (M+, 6.85), 314 (3.14), 240 (9.97), 224 (55.11), 136 (23.15), 124 (10.32), 105 (100), 77 (95.51), 57 (20.59); FT-IR cm^{-1} 2955, 2921 (ring CH_2), 1734, 1718 (carbonyl), 1616, 1601 (aromatic), 1457 (alkyl), 1447 (OCH_3), 1437 (CH_2-N); ^1H NMR(300 MHz,$CDCl_3$) δ 0.9 (t,3H,N-CH_2-CH_2-CH_2-**CH_3**), 1.7 (m,2H,N-CH_2-CH_2-**CH_2**-), 2.2 (m,2H,N-CH_2-**CH_2**-CH_2),2.6 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.3 (m,2H,C_5-H,C_1-H), 3.6 (s,3H,OCH_3), 5.1 (m,1H,C_3-H), 7.2-8.1 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{19}H_{25}NO_4$: C,69.56; H,7.82; N,4.05. Found: C,68.87; H,7.83; N, 4.26.

[1R-(*exo,exo*)-3-benzoyloxy-8-benzyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Ve): MP 96 °C, 25% yield (hexane); GC/MS m/z 379 (M+, 3.51), 348 (1.06), 258 (16.53), 170 (4.49), 105 (20.27), 91 (100), 77 (19.71); FT-IR cm^{-1} 1750, 1717 (carbonyl), 1278 (C-N), 1115 (C-O), 1034 (C-N), 714 (aromatic); ^1H NMR (300 MHz,$CDCl_3$) δ 1.3 (s,2H,N-CH_2-), 2.6 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.2 (m,2H,C_5-H,C_1-H), 3.6 (s,3H,OCH_3), 5.1 (m,1H,C_3-H), 7.2-8.1 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{23}H_{20}NO_4$: C, 72.82; H, 6.59; N, 3.69. Found: C, 72.66; H, 6.72; N, 3.65.

Procedure for N-Acylation



norcocaine

R=methyl
ethyl
propyl
phenyl

Scheme 5

Norcocaine (V)(898 mg, 3 mmol) was dissolved in a minimum amount of CH_2Cl_2 contained in a 50 mL Erlenmeyer flask. Triethylamine (1.5 mL) was added and the flask

was fitted with a rubber septum and magnetic stirrer. The solution was cooled to 0 °C, and the appropriate acid chloride (4.23 mmol) added *via* syringe over 6 to 10 min. The reaction mixture was allowed to stir at 0°C under N_2 for 4 hr. The mixture was then allowed to come to room temperature and stir for 48 hr. The mixture was washed with 10 mL of 2N HCl and again with 10 mL of distilled water. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The products were recrystallized from hot hexane. The acid chlorides utilized in this reaction procedure were acetyl chloride, propionyl chloride, butyryl chloride and benzoyl chloride.

ANALYTICAL:

[1R-(*exo,exo*)]-3-benzoyloxy-8-acetyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Vf): MP 138 °C, 32% yield (hexane); GC/MS m/z 331 (M+, 4.11), 288 (4.11), 225 (4.11), 210 (16.10), 194 (8.23), 168 (17.78), 136 (5.97), 105 (79.45), 77 (100), 59 (7.48); FT-IR cm^{-1} 1740, 1734 (ester carboxyls), 1672 (amide I, 3° amide), 1616,1601 (aromatic), 1234 (methyl ester), 1223 (amide II), 1204 (C-N); ^1H NMR(300 MHz,$CDCl_3$) δ 1.9 (s,3H,$COCH_3$), 2.3 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.7 (s,3H,OCH_3), 4.1 (m,2H,C_5-H,C_1-H), 5.3 (m,1H,C_3-H), 7.2-7.5 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{18}H_{21}NO_5$: C, 65.25; H, 6.34; N, 4.22. Found C, 65.63; H, 6.59; N, 4.73.

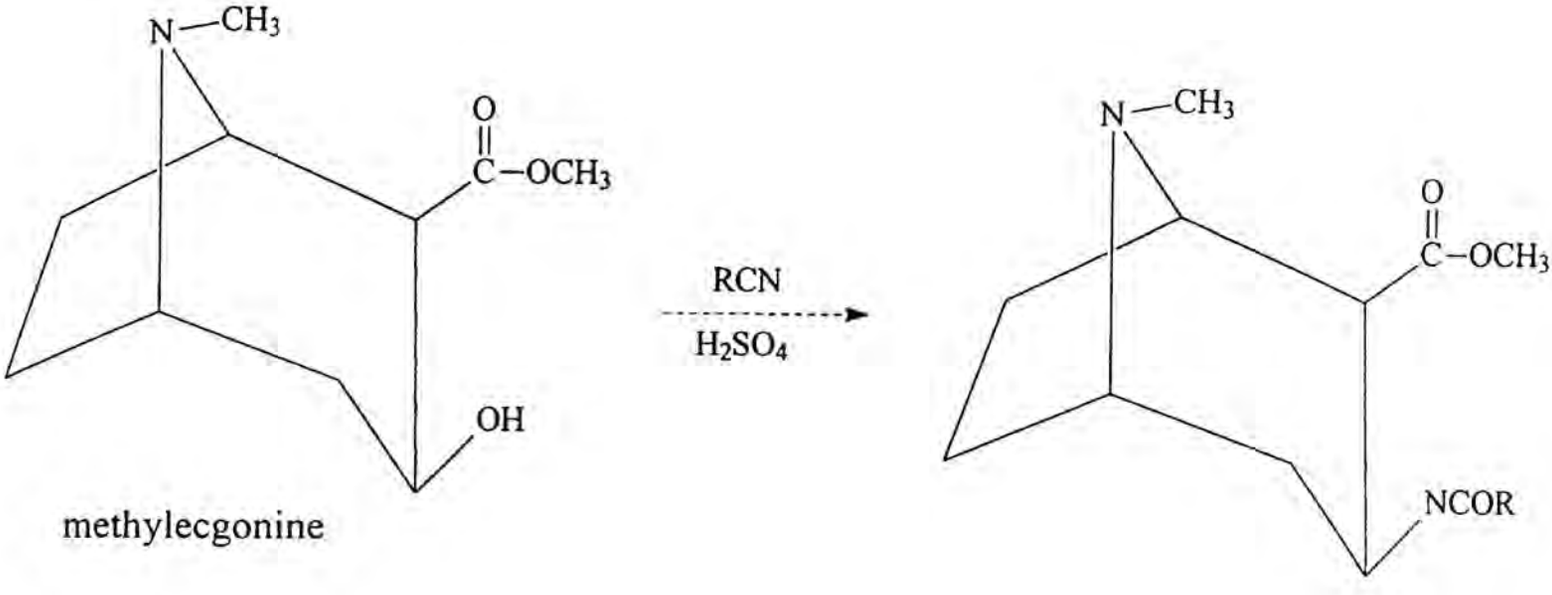
[1R-(*exo,exo*)]-3-benzoyloxy-8-propionyl-8-azabicyclo[3.2.1]octane-2-carboxyliacid, methyl ester (Vg): oil 39% yield; GC/MS m/z 288 (1.05), 212 (1.47), 168 (17.79), 136 (8.57), 105 (100), 77 (41.27); FT-IR cm^{-1} 1740, 1734 (ester carboxyls), 1672 (amide I, 3° amide), 1616,1601 (aromatic), 1234 (methyl ester), 1223 (amide II), 1204 (C-N); ^1H NMR(300 MHz,$CDCl_3$) δ 1.7 (t,3H,-CH_3), 1.9 (q,2H,-$COCH_2$-), 2.3 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.7 (s,3H,OCH_3), 4.2 (m,2H,C_5-H,C_1-H), 5.3 (m,1H,C_3-H), 7.2-7.5 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{19}H_{23}NO_5$: C, 66.08; H, 6.66; N, 4.06. Found: C, 66.26; H, 6.66; N, 4.71.

[1R-(*exo,exo*)]-3-benzoyloxy-8-butyryl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Vh): oil 31% yield; GC/MS m/z 359 (M+, 1.37), 329 (1.37), 253 (1.37), 179 (1.37), 135 (13.70), 107 (12.33), 105 (100), 77 (58.36), 59 (4.11); FT-IR cm^{-1} 1740, 1733 (ester carboxyls), 1671 (amide I, 3° amide), 1616, 1600 (aromatic), 1234 (methyl ester), 1223 (amide II), 1204 (C-N); ^1H NMR(300 MHz,$CDCl_3$) δ 1.7 (t,3H,-CH_3), 1.8 (m,2H,-CO-$\mathbf{CH_2}$-CH_2), 1.9 (q,2H,-$COCH_2$-), 2.3 (m,1H,C_4-H), 3.0 (m,1H,C_2-H), 3.7 (s,3H,OCH_3), 4.1 (m,2H,C_5-H,C_1-H), 5.3 (m,1H,C_3-H), 7.2-7.5 (m,5H,Ar-H); Elemental Analysis Calcd. for $C_{19}H_{23}NO_5$: C, 66.85; H, 6.96;N, 3.89. Found: C, 66.65; H, 6.99; N,3.81.

[1R-(*exo,exo*)]-3-benzoyloxy-8-benzoyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid, methyl ester (Vi): oil 27% yield; GC/MS m/z 289 (15.07), 272 (4.11), 184 (36.99), 168 (17.81), 152 (2.74), 136 (6.85), 105 (100), 77 (68.49), 59 (12.33); FT-IR cm^{-1}

1740, 1734 (ester carbonyls), 1672 (amide I, 3° amide), 1616, 1601 (aromatic), 1234 (methyl ester), 1223 (amide II), 1204 (C-N); ^{1}H NMR(300 MHz,$CDCl_3$) δ 2.0 (s,2H,-$COCH_2$-), 2.3 (m,1H,C_4-H), 3.3 (m,1H,C_2-H), 3.7 (s,3H,OCH_3), 5.3 (m,1H,C_3-H), 5.5 (m,2H,C_5-H,C_1-H), 7.2-8.0 (m,10H,Ar-H); Elemental Analysis Calcd. for $C_{23}H_{23}NO_5$: C, 70.23; H, 5.85; N, 3.56. Found: C, 70.30; H, 5.95; N, 3.53.

AMIDE FORMATION



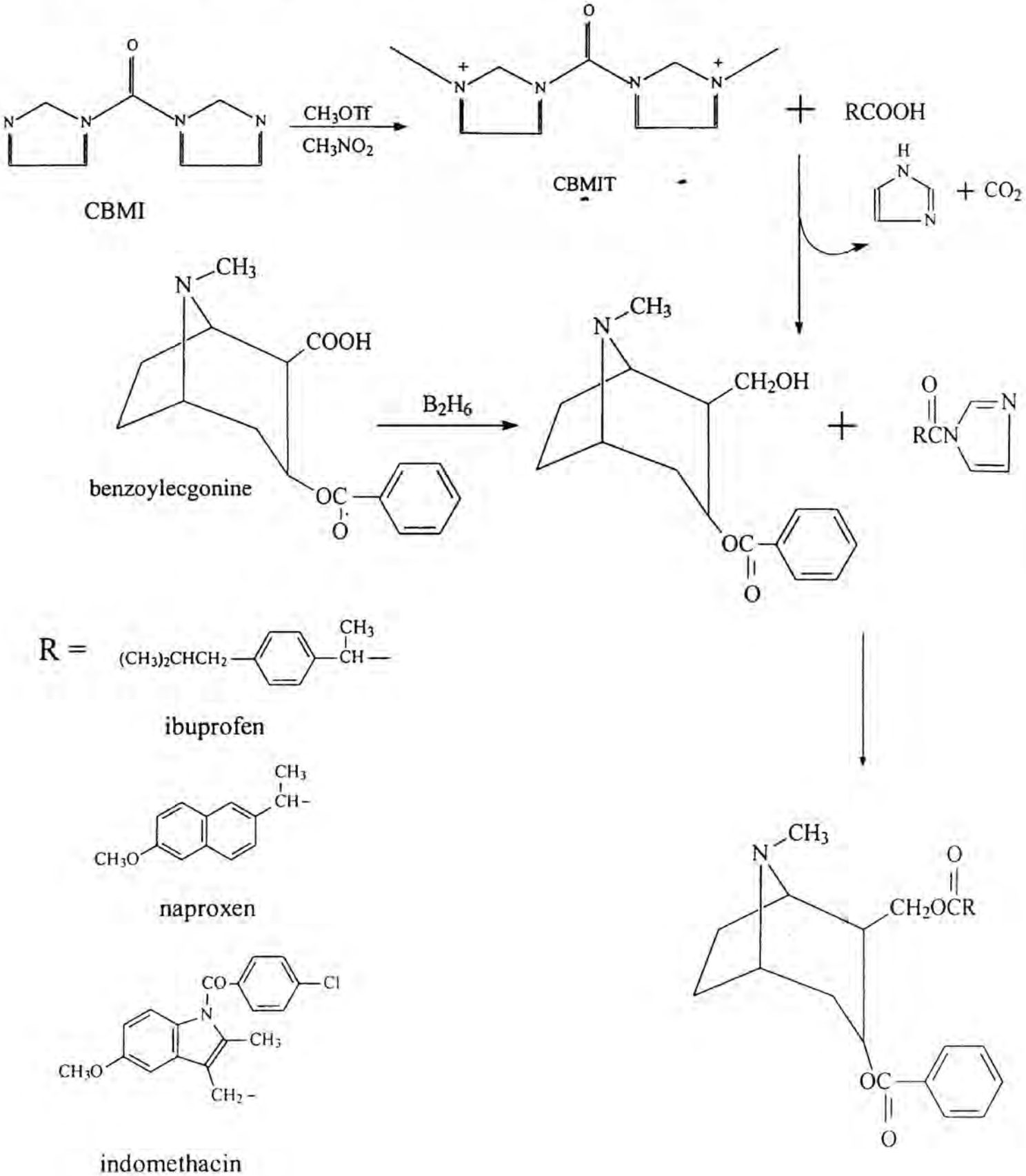
methylecgonine

Scheme 6

Methylecgonine (II)(2.00 g, 10 mmol) was suspended in 50 mL of the appropriate nitrile in a 100 mL Erleymeyer flask with warming and stirring. The solution was cooled to 0 °C and concentrated H_2SO_4 (6 mL) added dropwise over 15 min. The reaction was warmed to room temperature and allowed to stir overnight under N_2. A solution of freshly prepared 10% KOH was added in 10 mL increments and the pH monitored with litmus until it reached 12.0. The solution was poured over 300 g ice. A precipitate formed upon stirring which was later identified as an inorganic compound, possibly $KHSO_4$. The solution was filtered and the precipitate placed under vacuum. The filtrate was evaporated to dryness under reduced pressure and ether added to the residue. A precipitate formed and the solution was filtered, and this precipitate dried under vacuum. The solution was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness under the hood. GC/MS was performed on the residue and filtrates. No product formation was indicated, and the second filtrate proved to be ME (II).

BENZOYLECGONINE DERIVATIVES

Synthesis of 2β-Hydroxymethyl-3β-benzoyloxytropane (BOH) (VI)



Scheme 7

Benzoylecgonine (III)(1.00 g, 3.5 mmol) was dissolved in a minimum amount of dry acetronitrile contained in a 50 mL Erlenmeyer flask fitted with a magnetic stirrer and rubber septum. The flask containing the mixture was placed in an ice bath at 0°C under N_2 and 1.0 M diborane in THF (12.5 mL) was added slowly over 6 to 10 min *via* syringe. The solution was stirred at 0°C for 2 hr, then allowed to come to room temperature, and stirred overnight. Excess diborane was destroyed by careful addition of MEOH. The solution was concentrated by evaporation under reduced pressure, and the remaining solution made acidic to litmus by addition of 6N HCl. The solution was then evaporated to dryness under reduced pressure and allowed to cool to room temperature. The residue was dissolved in a minimum of CH_2Cl_2 and was made basic to litmus with 6N NH_4OH. The mixture was separated, the organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. GC/MS indicated that the oily residue was analytically pure product. The tan solid alcohol (934.8 mg, 98% yield) crystallized upon standing in a drying cabinet.

ANALYTICAL:

2β-Hydroxymethyl-3β-benzoyloxytropane (VI): MP 85 °C (no literature MP reported) 98% yield (hexane); GC/MS m/z 275 (M+, 12.33), 170 (6.85), 154 (100), 105 (32.88), 94 (34.25), 77 (34.25).

Since the compound had been previously synthesized and structurally elucidated, no further analytical procedures were done.

Synthesis of the Esters of 2β-Hydroxymethyl-3β-benzoyloxytropane

(See Scheme 7)

The reaction conditions were identical to those described in the attempt to synthesize ME esters using the carbonyldiimidazole procedure. The following quantities of reactants were used: 0.02 mL (0.18 mmol) CH_3OTf, 30.8 mg (0.20 mmol) carbonyldiimadazole, 49.0 mg (0.18 mmol) BOH (VI), and 0.57 mmol ibuprofen, naproxen, or indomethacin. GC/MS performed after 2 hr indicated that all of the BOH (VI) had reacted. After allowing the reaction to run overnight, there was no change in the GC/MS. After working up the reaction as described with the carbonyldiimidazole procedure, solid probe GCMS showed results consistent with a single component sample corresponding to the expected product. Each of the products were oils.

ANALYTICAL:

2-(4-isobutylphenyl)propionic acid, (3-benzoyloxy-2-tropyl)methyl ester (VIa): oil 22% yield; GC/MS m/z 463 (M+, 2.71), 258 (60.44), 161 (20.49), 136 (16.32), 118 (14.11), 105 (43.46), 104 (4.24), 94 (29.98), 91 (15.49), 82 (100), 77 (23.48), 57 (9.89); FT-IR cm^{-1} 2955, 2921 (ring CH_2), 1740, 1734 (ester carbonyls), 1616, 1601 (aromatic), 1457 (alkyls), 668 (aromatic); ^1H NMR (300 MHz, $CDCl_3$) δ 0.9 (d, 6H, CH-(**CH_3**)$_2$), 1.5 (d, 3H, -CH_3), 1.8 (m, 1H, CH_2**CH**(CH_3)$_2$), 2.3 (s, 3H, N-CH_3), 2.4 (m, 2H, Ar-CH_2), 2.5 (m, 1H, C_4-H), 3.1 (m, 1H, C_2-H), 3.3 (m, 2H, C_5-H, C_1-H), 3.5 (d, 2H, -CH_2OCO-), 3.6 (d, 1H, COC**H**-), 5.2 (m, 1H, C_3-H), 7.8-8.1 (m, 9H, Ar-H).

2-(6-methoxy-naphthyl)propionic acid, (3-benzoyloxy-2-tropyl)methyl ester (VIb): oil 26 % yield; GC/MS m/z 487 (M+, 1.45), 258 (35.81), 229 (26.20), 185 (100), 154 (13.72), 153 (15.45), 136 (15.96), 126 (3.35), 105 (30.80), 94 (27.08), 77 (15.10); FT-IR cm^{-1} 2955, 2920 (ring CH_2), 1740, 1734 (ester carbonyls), 1617, 1601 (aromatics), 1260 (ether); ^1H NMR (300 MHz, $CDCl_3$) δ 1.5 (d,3H, $-CH_3$), 2.3 (s, 3H, N-CH_3), 2.5 (m, 1H, C_4-H), 3.1 (m, 1H, C_2-H), 3.3 (m, 2H, C_5-H, C_1-H), 3.5 (d, 2H, $-CH_2OCO$), 3.6 (d, 1H, -COCH-), 3.7 (s,3H, OCH_3) 7.8-8.1 (m, 11H, Ar-H).

1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid (3-benzoyloxy-2-tropyl)methyl ester (VIc): oil 20% yield; GC/MS m/z 614 (M+, 0.38), 356 (3.15), 258 (29.60), 139 (100), 136 (11.31), 111 (24.01), 94 (15.13) FT-IR cm^{-1} 3200 (indole N-H), 2955, 2920 (ring CH_2), 1740, 1734 (ester carbonyls), 1670 (amide I) 1617, 1600 (aromatics), 1260 (ether), 1220 (amide II); ^1H NMR (300 MHz, $CDCl_3$) δ 2.1 (s, 3H,-CH_3), 2.3 (s, 3H, N-CH_3), 2.5 (m, 1H, C_4-H), 3.1 (m, 1H, C_2-H), 3.3 (m, 2H C_5-H, C_1-H), 3.5 (d, 2H, CH_2OCO), 3.6 (s, 2H, $COCH_2$), 3.7 (s,3H, OCH_3), 7.8-8.1 (m, 13 H, Ar-H).

DISCUSSION OF SYNTHETIC RESULTS

The original research plan proposed the synthesis of derivatives of ecgonine involving esterification of and amide formation at the three position, and alkylation and acylation of the eight position. These compounds were to be synthesized from ecgonine (IV)(EC), norecgonine (NEC), or methylecgonine(II) (ME). Therefore the initial reactions were attempted with these goals in mind.

ESTERIFICATION

The esterification of the 3-OH of ME (II) was the first series of reactions that was attempted. The initial difficulty encountered in the synthesis of the ester series was the neutralization of methylecgonine HCl (ME HCl) to produce the free base. This was necessary because a proton source in the reaction mixture would deactivate the acidic reactants such as the acid chlorides or anhydrides. Due to the relative insolubility of the free base (ME) (II) in organic solvents, the standard neutralization of tropane salts such as the neutralization of cocaine HCl with KOH was not entirely successful. Unlike cocaine (I), methylecgonine (II) does not readily precipitate from aqueous solution. Consequently, the recovery yield of the ME (II) free base was only 15-30% compared with 90-98% for cocaine (I). Although the yield was relatively low, GC/MS data

confirmed that the ME(II) obtained was analytically pure. This product was used as the starting material in attempts to synthesize ester and amide derivatives of ecgonine (IV).

Attempts were made to synthesize a series of short chain aliphatic acid esters, as well as two salicylate esters of the 3-hydroxy group of ME (II). Acid anhydrides or acid chlorides were utilized as the acyl donor groups in the attempted esterifcations. In addition to its limited solubility, the requirement of an aprotic solvent posed another problem in the esterification of ME (II). Dry benzene (10) has been used in the esterification of ME (II) to cocaine (I) with either benzoyl chloride or benzoic anhydride. However, the ME (II) obtained from the above neutralization procedure proved insoluble in benzene even at high temperatures. Other solvents evaluated for ME (II) solubility included acetonitrile, hexane, dioxane, tetrahydrofuran, toluene, ethyl acetate, dimethylsulfoxide, dimethylformamide, nitromethane, chloroform, and methylene chloride. Of these, only nitromethane solubilized the ME (II) to any extent.

Vogel (52) describes the use of acid chlorides and anhydrides as being an effective synthetic method for esters in which the alcohol of interest is either primary or secondary. The 3-*beta*-OH of ME (II) is secondary and several reactions were attempted with this scenario. Specifically, a total of seven attempts were made with butyric anhydride and acetic anhydride in two separate reaction procedures. Another seven reactions were run using either acetylsalycyloyl chloride (3), salicyloyl chloride (2), and butyryl chloride (2).

In the first procedure utilizing the anhydrides, acetonitrile was used to suspend the ME (II), and pyridine was the agent used as a catalyst ($k_b = 1.4 \times 10^{-9}$) for the reaction.

Once the reaction was removed from heat and cooled, water was added. The azeotropic mixture formed with the pyridine was then removed by evaporation under reduced pressure. Aqueous sodium bicarbonate was added to the residue to neutralize the free carboxylic acid. The presumed product was then extracted from this aqueous mixture into ether and the ether was allowed to evaporate under the hood. In the second procedure, nitromethane was utilized and the solvent and triethylamine ($k_b = 5.65 \times 10^{-5}$) as the catalyst. Thus both the solubility of the starting material and the strength of the catalyst were increased. The reaction was allowed to remain at room temperature under nitrogen. The subsequent work up was the same as that used in the first procedure, with additional drying of the organic extracts using anhydrous Na_2SO_4. The GC/MS results indicated that in all of the reaction attempts, only minute amounts of the acetate ester formed in a quantity insufficient for further analytical evaluation.

The procedure utilizing the acid chlorides was a standard esterification in which ethyl acetate was employed as the reaction medium and pyridine was utilized as the catalyst and to neutralize the hydrogen chloride formed during the reaction. The presence of minute quantities of each product were verified *via* GC/MS in each of the seven reactions attempted using acetylsalicyloyl chloride, salicyloyl chloride, or butyryl chloride; however, no product was present in a quantity sufficient for further analytical determination.

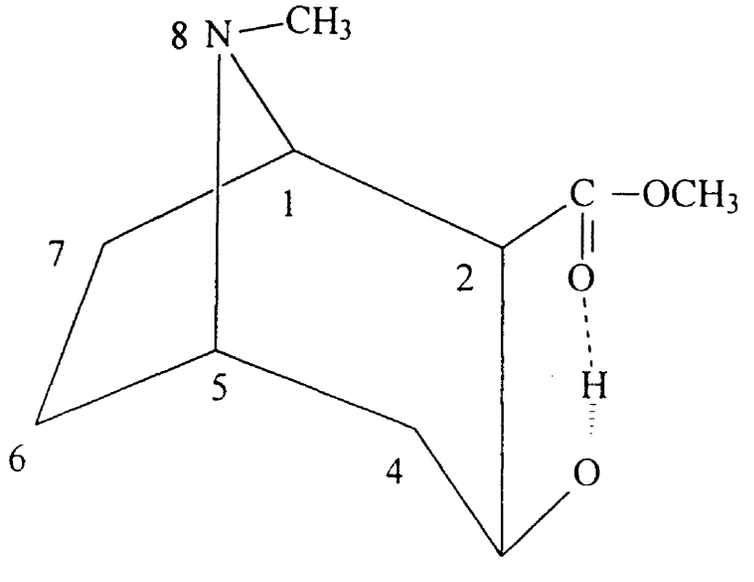
As it became obvious that conventional methods of esterification would not be productive, a search for an effective approach was conducted. Paul and Anderson (53,54) reported that N,N'-carbonyldiimidazole was useful in peptide formation. Saha

(55) took this one step further with the *bis*-alkylation of carbonyldiimidazole by methyl triflate to form 1,1'-carbonyl-*bis*-(3-methylimidazolium)triflate (CBMIT). The use of this latter reagent virtually eliminated the problems associated with peptide and ester formation, such as racemization, complex reagents, or side-reactions. The highly reactive acyl imidazolium ion intermediate facilitates the reaction. It readily donates the acyl group to the peptide fragment, amino acid, or alcohol to yield peptides, amides, or esters. The best solvent found for this reaction is nitromethane. This eliminated the problem of solubility associated with ME (II).

The products of each of these esterfication attempts were analyzed using GC/MS. For the reactions in which acetic, propionic and butyric acids were used, the spectrum indicated the formation of a very small amount of the desired product, and that the remaining material was unreacted ME (II). Using preparative thin layer chromatography, only the propionic acid ester of ME (IIc) was isolated and only in a quantity (approximately two mg) sufficient to perform GC/MS analysis proving that particular band was the propionic acid ester. In the reactions using salicylic and acetylsalicylic acids, the GC/MS revealed a complex mixture of products including small amounts of each of the desired esters, the free acids, the acetate ester of methylecgonine, and the starting material, ME (II). The GC/MS analysis indicated that none of the mixtures contained enough of the desired products to attempt separation. In the acetylsalicyloyl acid reaction, a transesterification of the 3-*beta*-OH of ME (II) by the highly reactive acetyl ester group in the acetylsalicylic acid competed with the desired esterification of that group with the carboxylic acid moiety on the salicylate. GC/MS evidence indicated

that the major product was the acetyl ester of ME (Ve) which formed in a ratio of nearly 10:1 over the desired acetylsalicyloyl ester (Vk). Neither product formed in quantities sufficient for structural verification.

Despite the utilization of activated acids, acid derivatives, or carbonyl transfer groups, the reactions in which the esterification of the 3-*beta*-OH was of interest were quite unreactive. The relative lack of solubility of the ME (II) alone could not account for these results. A molecular model evaluation of the ME (II) molecule revealed a high probability for hydrogen bonding between the 3-*beta*-OH hydrogen and the carbonyl oxygen of the 2-*beta*-methyl ester (Fig. 8). This phenomenon would greatly decrease the



methylecgonine

Fig. 8

reactivity of the hydroxyl group toward esterification by reducing the availability of the electrons of the oxygen for attack on the carbonyl carbon of the acyl species and would therefore explain why these reactions produced less than optimum results.

In an attempt to synthesize and isolate any ecgonine derivative of any of the esters from the crude reaction mixtures, a procedure similar to that of Bell and Archer (56) for the synthesis of benzoylecgonine (III) from cocaine (I) was utilized. The reaction was followed by GC/MS to minimize the formation of ecgonine (IV) and ecgonidine which were undesired side products of the reaction. Of all these reaction attempts, only the butyrate ester (IVb) was formed in any isolatable quantity.

N-DEMETHYLATION

An initial search of the literature involving the N-demethylation of cocaine (I) or benzoylecgonine (III) revealed two major approaches. Findlay's (57) method utilizing potassium permanganate oxidation of benzoylecgonine (III) was a relatively facile procedure and produced an 82% yield of norbenzoylecgonine HCl. Stenberg (58) described an improved method of preparing norcocaine (V) from cocaine (I) by controlling the pH while using the same type oxidation employed by Findlay. Maintaining an acidic pH was necessary to prevent the acyl transfer of O-acetyl and O-benzoyl groups to the 2° amine that occurs in basic media (59) in similar tropane systems. Since the N-modified ecgonine derivatives of these compounds were of interest, it was determined that attempting the reaction with benzoylecgonine (III), while controlling the pH, would enhance the yield, and eliminate an additional hydrolysis step.

In each of the ten reactions attempted, the ^1H NMR spectrum of the product contained the N-CH_3 peak located at δ 2.2 ppm, and there was no evidence of the presence of the desired nor-methyl product. The remainder of the ^1H NMR spectrum was consistent with that of the starting material and there was no indication of a mixture of benzoylecgonine (III) and nor-benzoylecgonine.

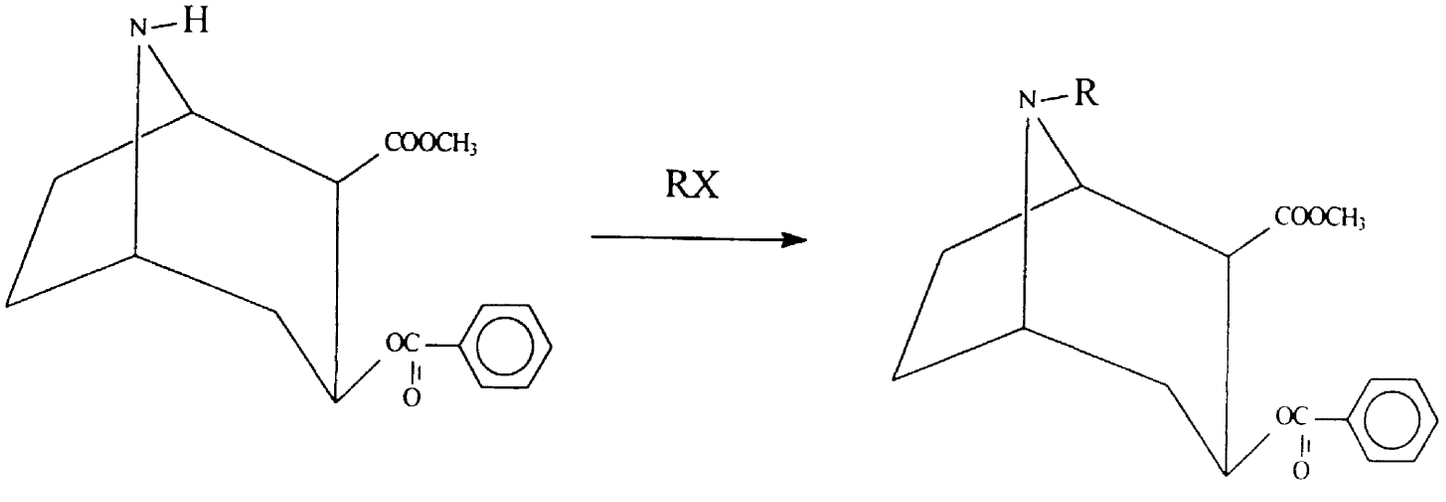
Baldwin (60) developed a reaction method by which direct demethylation of cocaine (I) was accomplished using chloroformate esters. He reported an 85% yield of the norcocaine HCl when using 2,2,2,-trichloroethyl chloroformate and a 55% yield of the norcocaine (V) when vinyl chloroformate was employed. Borne (61), utilizing a similar reaction sequence with 2,2,2-trichloroethyl chloroformate, reported a yield of 56% of the norcocaine HCl. However, both these methods were very time consuming and required several manipulations of the reaction mixture such as hydrolysis of the intermediate carbamate using zinc dust and several extraction procedures. With the expense of the starting material in mind, the search continued for a better approach.

Meltzer and co-workers (62) utilized α-chloroethyl chloroformate (ACE-Cl) in their N-demethylation reaction with impressive results (74% norcocaine (V)) requiring a minimum of time and manipulation. The conditions for the hydrolysis of the carbamate intermediate were appropriate for use in the presence of such sensitive functional groups as the esters on the C-2 and C-3 positions of the tropane ring. However, in an effort to further improve the yield, this method was modified by separating the unreacted cocaine (I) from the intermediate carbamate *via* an extraction into acidic water prior to the hydrolysis of the carbamate with methanol. As a result, a 96% yield of the norcocaine

base (V) was obtained. The structural identity of the compound was confirmed by GC/MS, FT-IR, and ^{1}H NMR. The characteristic pattern of this tropane in the GC/MS included peaks at 59 ($COOCH_3$), 77 (C_6H_5), 168 (1-methyl-1-pyrolinium ion), and 289 (M+). The ^{1}H NMR confirmed the absence of the N-methyl peak in the area of δ 2.8 ppm. The FT-IR confirmed the presence of the secondary amine (1275 cm^{-1}).

N-ALKYLATION

Historically, alkylation of the tropane nitrogen (Scheme 8) has been accomplished *via* reaction with alkyl halides. Lazer (63) synthesized several derivatives including



Direct N-alkylation

Scheme 8

N-cylopropylmethylnorcocaine, N-allylnorcocaine, and N-dimethylallylnorcocaine.

Millius (64) synthesized and characterized a series of N-substituted 3-(4-flurophenyl)tropane derivatives. Abraham (65) also reported the synthesis of a group of N-modified cocaine derivatives, one of which was N-benzylnorcocaine. Although each of the procedures cited above varied in such parameters as the type of catalyst used, the reaction time, and/or the solvent system, all results were similar, and all required the use of flash chromatography for purification. Also the risk of quaternary ammonium salt formation was significant, creating further difficulties in separation and reduction of yield. To eliminate the necessity of column chromatography, the possibility of quaternization, and to maximize the yield, reductive alkylation was investigated. Vogel (66) describes the process by which a primary or secondary amine is reductively alkylated under catalytic conditions in an ethanol solution using hydrogen under pressure and Raney nickel. The use of sodium borohydride is also indicated as an effective *in situ* reducing agent when primary amines are mixed with aliphatic aldehydes or ketones. Further investigation (67,68) revealed the use of sodium cyanoborohydride as a selective reducing agent for a given functional group in the presence of other sensitive functional groups. This reagent appeared ideal for use with the cocaine (I) molecule with its susceptible ester groups. Another advantage of the sodium cyanoborohydride molecule lies in the strongly electron-withdrawing properties of the cyano group, creating a milder and more selective reducing agent than sodium borohydride (Fig. 32). By following the progress of the reactions to completion with GC/MS, the use of flash chromatography was eliminated and recrystallization was utilized for purification. The procedure utilized

for this series of reactions was a modification of a synthetic scheme originally described by Oka (69).

The GC/MS spectrum contained the expected parent ion corresponding to the molecular ion (M+) of each product and/or fragmentation patterns typical of tropanes (59, $COCH_3$; 77, C_6H_5; 168, 1-methyl-1-pyrolinium ion), the ^1H NMR spectra verified the appearances of the appropriate patterns for the respective N-substituents at the expected chemical shifts such as the quartet-triplet (δ 2.3, 1.0 ppm) indicative of the N-ethyl derivative (Va), the triplet-multiplet-triplet (δ 2.3, 1.8, 1.0 ppm) of the N-propyl derivative (Vb), the multiplet-singlet (δ 2.2, 0.8-1.0 ppm) of the N-isopropyl compound (Vc), the triplet-multiplet-multiplet-triplet (δ 2.2, 2.0, 1.7, 0.9 ppm) of the N-*n*-butyl compound (Vd); and, for the N-benzyl product (Ve), the increased integration of the aromatic region (δ 7.2-8.1 ppm) and the new singlet for the N-CH_2 at δ 1.3 ppm. The FT-IR indicated the expected disappearance of the secondary amine band (1275 cm^{-1}) present in the starting material and expected absorbances characteristic of the functional groups in the desired products. Specifically, there was an increase in the aliphatic C-H bands. These products included N-ethylnorcocaine (Va), N-*n*-propylnorcocaine (Vb), N-isopropylnorcocaine (Vc), N-*n*-butylnorcocaine (Vd), and N-benzylnorcocaine (Ve).

N-ACYLATION

The procedure used in this series of reactions was a standard amino-acylation reaction as discussed in March (71). A similar reaction sequence was reported by Abraham (65). The procedure is highly exothermic, and the temperature was controlled

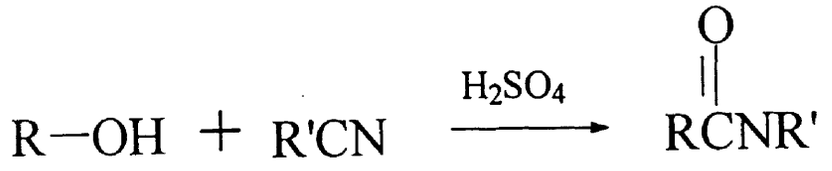
with cooling to 0 °C. Triethylamine served to neutralize the HCl liberated by the reaction.

The results for this series of reactions were similar to the N-akyl analogs. The products were characterized using GC/MS, FT-IR, ^{1}H NMR with support from elemental analysis. The GC/MS results indicated the expected parent ion and/or fragmentation pattern characteristic of these tropane derivatives (59, $COCH_3$; 77, C_6H_5; 168, 1-methyl-1-pyrolinium ion). The ^{1}H NMR data depicted the singlet (δ 1.9 ppm) of the acetyl group of the N-acetyl derivative (Vf), the quartet-triplet (δ 1.9, 1.7 ppm) of the N-propionyl group of that derivative (Vg), the triplet-multiplet-triplet (δ 1.9, 1.8, 1.7 ppm) of the N-butyryl group of that product (Vh); and the increased integration of the aromatic multiplet (δ 7.2-8.0 ppm) and multiplet (δ 2.0 ppm) of the benzoyloxy component of that product (Vi). [Each of the above characteristics of these spectra indicated the downfield shift expected in a comparison of a series of amines with the comparable amides]. In each instance, the FT-IR confirmed the presence of the amide I (1672 cm^{-1}) and II (1222 cm^{-1}) bands, as well as the other functional groups expected. The products included N-acetylnorcocaine (Vf), N-propionylnorcocaine (Vg), N-butyrylnorcocaine (Vh), and N-benzoylnorcocaine (Vi).

AMIDE FORMATION

The Ritter reaction (72) (Scheme 9) is a standard reaction utilized in the formation of amides from alcohols. This procedure appeared to be satisfactory for the purpose of synthesizing the 3β-amide derivatives of ecgonine (IV). Secondary alcohols

will react with nitriles with the use of concentrated H_2SO_4 and relatively polar solvents such as the nitrile of interest may be utilized. This would overcome the solubility concerns associated with ME (II). However, the hydrogen bond formation between the 3β-OH and the carbonyl oxygen of the 2β-ester described in the discussion on ester synthesis may have contributed to the observed lack of reactivity.



Ritter reaction

Scheme 9

BENZOYLECGONINE DERIVATIVES

Analysis of the product (Esterom®) that is under clinical trials revealed the presence of BE (III) and related compounds. In addition to the proposed esterification of the 3-OH with selected NSAIDs, it was deemed feasible to couple the BE (III) molecule in a pro-drug fashion with these NSAIDs. Consequently, it was decided to combine the reduced BE (III) molecule *via* esterification with three commonly used NSAIDs: ibuprofen, naproxen, and indomethacin. The first step was to convert the 2-carboxylic acid group of BE (III) into a primary alcohol. This primary alcohol could

then be esterified with the appropriate NSAID to yield a "double" prodrug, with the BE *(III) moiety possessing the activity of the product in the clinical trials and the NSAID* portion of the molecule having its anti-inflammatory activity.

A procedure similar to the one reported by Lewin (74) was utilized in the synthesis of the starting material (BOH) (IIIa) of this series of compounds. The procedure utilizing carbonyldiimidazole as an activated acyl carrier discussed in the section on attempted esterification was the reaction of choice in this situation as the molecules involved contained multiple reactive groups which increased the potential of complicated side reactions. The reactions were followed with GC/MS, which facilitated optimum product formation as evidenced by the disappearance of the starting material. Solid probe GC/MS was necessary to determine the presence and purity of the final products due to the lack of volatility of the compounds. In each case, the results were consistent with a single component and depicted the expected fragmentation and M+ peaks.

BIOLOGICAL EVALUATION

BACKGROUND

The Medications Development Division (MDD) of the National Institute of Drug Abuse (NIDA) has established a Cocaine Treatment Discovery Program (CTDP) to identify potential treatments for the medical management of cocaine abuse. This protocol is designed to identify compounds which either substitute for cocaine or antagonize cocaine's effects. The screening components of this program consist of initial *in vitro* biochemical assays of each compound. Compounds demonstrating appropriate *in vitro* activity then are utilized in *in vivo* pharmacological studies of mouse locomotor activity and rat drug discrimination and self-administration. Table 2 summarizes the procedure for each component of the CTDP screening.

IN VITRO METHODS

Each of the N-modified compounds were tested for their effects on radioligand ([^{125}I]RTI-55) binding to and [^{3}H]dopamine uptake by C6 cells expressing the cDNA for the human dopamine transporter (C6-hDAT cells).

[^{125}I]RTI-55 Binding:

Cell Preparation: Cells were grown on 150 mm diameter tissue culture dishes. The medium was poured off the plate; the plate washed with 10 mL of phosphate buffered saline and 10 mL of lysis buffer (2 mM HEPES, 1 mM EDTA) was added. After 10 min, the cells were scraped from the plates, poured into centrifuge tubes and centrifuged for 20 min at 30,000 x *g*. The supernatant was removed, and the pellet was resuspended in 20 mL of 0.32 M sucrose with a Polytron at setting 7 for 10 sec.

Assay: Each assay sample contained 50 μL of the membrane (cell) preparation (approximately 50 μg protein), 25 μL of drug (from a solution of the drug dissolved in a minimum of MEOH), and 25 μL of [^{125}I]RTI-55 (40-80 pMol) in a final volume of 250 μL. Krebes HEPES was used for all assays. Membranes were preincubated with the compounds of interest for 10 min prior to addition of [^{125}I]RTI-55. The reaction was incubated for 90 min at room temperature in the dark and was terminated by filtration onto GF/C filters using a Tom-tech harvester. Scintillation fluid (50 μL) was added to each square and the radioactivity remaining on the filter was determined using a Wallac β-plate reader. Competition experiments were conducted with duplicate determinations. The data was analyzed using GraphPAD Prism, which converted IC_{50} values to K_i values.

[^{3}H]Neurotransmitter Uptake:

Cell Preparation: The cell preparation procedure for the [^{125}I-55] binding was utilized.

Assay: For experiments involving uptake of [^{3}H]DA, the medium was removed and Krebs HEPES buffer (25 mM HEPES, 122 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$,

2.5 mM $CaCl_2$, 1 μL pargyline, 0.2 g/100 mL glucose, 0.02 g/100 mL ascorbic acid, pH 7.4) was added. The uptake was initiated by the addition of [^{3}H]DA (20 mM, specific activity 20-53 Ci/mmol) in a final volume of 500 μL. Mazindol (5 μM) or imipramine (5 μM) was used to define nonspecific uptake. Cells are preincubated for 10 min with the compound of interest before the addition of the radioligand. Uptake was terminated after a 2 min incubation by removing the buffer. The plate was then placed on ice and washed twice with 1 mL phosphate-buffered saline that had been cooled in an ice bath. Trichloroacetic acid (TCA, 0.5 mL, 3%) was added to each well, the cells were allowed to sit for 15 min, and radioactivity in the TCA was determined by conventional liquid scintillation spectrometry.

TABLE 2

CTDP SCREENING AND PROFILING PROTOCOLS

TEST	PROTOCOL
[^{125}I]RTI-55 (CIT) TRANSPORTER BINDING	Only compounds with a rational structural basis for interacting at the dopamine transporter will be tested for [^{125}I]RTI-55 binding activity. Human cloned dopamine transporter cell membranes (200μl) are incubated in Krebs-HEPES buffer (25mM HEPES, 120mM NaCl, 5mM KCl, 2.5mM $Cacl_2$, 1.2mM $MgSO_4$, 1μM pargyline, 2mg/ml glucose, and 0.2mg/ml ascorbic acid) for 2 hours at 20°C with 25μl of test compound at 25μl of [^{125}I]RTI-55 (0.05nM final concentration). Nonspecific binding is determined by incubating in the presence of 5μM mazindol. Active compounds will then be tested for their ability to affect the rate of dopamine uptake *in vitro*.
LOCOMOTOR ACTIVITY (LMA)	The initial behavioral screen consists of testing compounds alone and in combination with cocaine on locomotor activity in mice. This is accomplished through the use of an automated photocell monitor system. The effects of the test compound alone on locomotor activity is evaluated first. In this experiment, sufficient doses are tested in order to determine maximum effects and ED_{50} values. For compounds which do not stimulate activity or are weak stimulant-type drugs (i.e. compounds which produce maximum stimulant effects less than cocaine), interaction studies will be conducted to determine the effect of the test compound on cocaine-induced locomotor activity. These compounds are tested for their ability to antagonize the stimulant effects of cocaine over an appropriate dose range. AD_{50} values are calculated, when possible.
DRUG DISCRIMINATION (DD)	The second tier test is drug discrimination. These experiments are conducted in standard 2-lever operant chambers. Depending on the results obtained from the locomotor activity studies, the test compound will either be tested for its ability to antagonize cocaine's discriminative stimulus effects or to generalize to the discriminative stimulus effects of cocaine. Drug discrimination antagonist studies are conducted when compounds demonstrate weak or no stimulant effects in locomotor activity studies. In this case, several doses of the test compound are tested in combination with the training dose of cocaine. drug discrimination substitution tests are conducted when compounds exhibit maximum stimulant effects which are equal to or greater than cocaine. For the substitution experiments, several doses of the test compound are tested for their ability to substitute for cocaine. Drug discrimination experiments are initially tested in rats. For those compounds which show a promising pharmacological profile, drug discrimination experiments in monkeys will be conducted.
SELF-ADMINISTRATION (SA)	The third tier test is self-administration. These experiments are conducted in standard operant conditioning chambers. Antagonism of the reinforcing effects of cocaine is examined by assessing changes in rates of cocaine self-administration after pretreatment with the test compound. Specificity of the effect is examined by comparing effects of the test compound on behavior maintained by cocaine and another reinforcer. At least two doses of the test compound shall be examined with two doses of cocaine. In selected cases, the ability of the test compound to maintain self-administration behavior is also assessed. Drug self-administration experiments are initially tested in rats. For those compounds which show a promising pharmacological profile, drug self-administration experiments in monkeys will be conducted.

RESULTS

The results are summarized in Table 3 below.

TABLE 3

RESULTS OF BIOLOGICAL ASSAYS

COMPOUND	[^{125}I]RTI-55 BINDING K_i (nM)	HILL CO-EFFI-CIENT	[^{3}H]DA UP-TAKE IC_{50} (nM)	HILL CO-EFFI-CIENT
COCAINE(I)	351±62	-0.98±0.08	240±2	-0.94±0.16
Va	2259±331	-0.90±0.19	2007±981	-1.40±0.62
Vb	>10μM			
Vc	>10μM			
Vd	494±53	-0.86±0.04	245±83	-0.63±0.03
Ve	3925±501	-0.73±0.16	1216±264	-0.84±0.12
Vf	>10μM			
Vg	>10μM			
Vh	>10μM			
Vi	7136±1828	-0.91±0.03	4645±1242	-1.32±0.36

The results of the *in vitro* studies did not justify further investigation of the compounds, and no *in vivo* evaluations were performed.

DISCUSSION

In the N-alkylated series of compounds, the N-ethyl, N-propyl, N-isopropyl, N-*n*-butyl, and N-benzyl derivatives have lower affinities for ([^{125}I]RTI-55) radioligand binding to C6-hDAT cells than cocaine, the standard compound, for the same site. In the uptake assays, with the exception of the N-*n*-butyl derivative, each of these compounds was less potent than cocaine in blocking the uptake of [^{3}H]dopamine. The N-*n*-butyl derivative was equipotent to cocaine in blocking the uptake of [^{3}H]dopamine.

All of the compounds in the N-acylated series including the N-acetyl, N-propionyl, N-*n*-butyryl, and N-benzoyl derivatives have lower affinities for ([^{125}I]RTI-55) radioligand binding to C6-hDAT cells than cocaine, the standard compound, for the same site. These compounds were also less potent than cocaine in blocking the uptake of [^{3}H]dopamine.

CONCLUSIONS

Chemistry

Esterification of the 3-*beta*-hydroxy of ME (II) proved to be infeasible using standard esterification techniques. Changes in the solvent, strength of the catalyst and the acylation agent (including the use of carbonyldiimidazole acyl activation) did not result in the formation of anything more than a negligible amount of any ester product as indicated from GC/MS analysis. No product could be isolated in a quantity that was adequate for comprehensive structural analysis. It is unlikely that the poor solubility of II alone could account for these results. An evaluation of a molecular model of II revealed a high probability for hydrogen bonding between the 3-*beta*-hydroxy hydrogen and the carbonyl oxygen of the 2-*beta*-methyl ester. This interaction would greatly decrease the reactivity of the hydroxy group to esterification and, when combined with the poor solubility of II, could explain the lack of success with these reaction attempts.

Attempts to form amides at the 3-*beta*-position of II using the Ritter reaction were also futile. Even though this reaction is a standard for the synthesis of amide from alcohols, the intramolecular hydrogen bonding described for II could also contribute to the lack of reactivity in this reaction as well as in the esterification reaction.

The N-demethylation of cocaine (I) or BE (III) using the $KMNO_4$ oxidation

method did not appear to be as facile as reported (57). However, modification of a procedure employing α-chloroethyl chloroformate to produce N-demethyl cocaine (V) proved efficient. The use of reductive alkylation to form N-alkyl derivatives of V produced satisfactory yields of compounds of analytical purity while avoiding the disadvantages of direct alkylation. Likewese, standard acylation procedures provided a series of N-acylated derivatives of V of satisfactory yields and purity.

The synthetic procedures leading to the formation of potential prodrugs for both III and the respective NSAID employed proved to be satisfactory. The reduction of III to its corresponding 2-*beta* primary alcohol, BOH (IIIa), was straightforward and efficient. The esterification of this primary alcohol with either ibuprofen, naproxen or indomethacin was accomplished using the carbonyldiimidazole activated acyl carrier and led to limited yields of each product. However, enough purified material of each product was isolated to obtain NMR, GC/MS and IR structural confirmation.

Biological Activity

For the N-alkyl series of norcocaine (V) derivatives, only the N-butyl (Vd) demonstrated *in vitro* activity. While Vd was equipotent with I in blocking the uptake of [^{3}H]dopamine, it had a significantly lower affinity than I for transporter binding and was not found to be suitable candidate for *in vivo* evaluation. While the significance of the activity of Vd is unclear from these results, it can be concluded that any increase in the size of the N-alkyl beyond the methyl group of I leads to a decrease in both transporter binding affinity and in [^{3}H]dopamine uptake. These results are consistent

with an earlier study (65) in which the replacement of the N-methyl group of cocaine with sterically larger groups reduced activity.

None of the N-acyl derivatives of V demonstrated meaningful transporter binding affinity nor [^{3}H]dopamine uptake activity. N-acylation of the secondary amine of V appears to irradicate meaningful transporter binding affinity and [^{3}H]dopamine uptake activity as well. The latter would be expected to be associated with the loss of basic character of the nitrogen and the related loss of an ionic attraction for the receptor. These results are consistent with those reported by Abraham for N-acetyl norcocaine(65). However, it is important to note that Kozikowski(48) has reported sulfonamide derivative of norcocaine that have similar and/or greater activity than cocaine in [^{3}H]mazindole binding and in [^{3}H]dopamine binding studies. From these results it can be concluded that, while the ionic attraction associated with the basic tertiary nitrogen of cocaine may contribute to its binding and subsequent activity, compounds containing a neutral nitrogen bridge can also possess a high binding affinity, which indicates that a basic nitrogen is not essential for this activity.

FUTURE RESEARCH

Priorities for future research include the following:

1. Testing the benzoylecgonine/NSAID derivatives in arthritic animal models.

2. Testing the N-alkyl and N-acyl benzoylecgonine derivatives in arthritic models and as cocaine antagonists.

3. Synthesizing additional benzoylecgonine/NSAID ester to include the more potent NSAIDs.

4. Continued efforts to synthesize 3-*beta*-analogs of benzoylecogonine to include its 3-*beta*-amide isoester.

5. Elucidation of the effects of various modifications of the cocaine nucleus on the cloned dopamine transporter.

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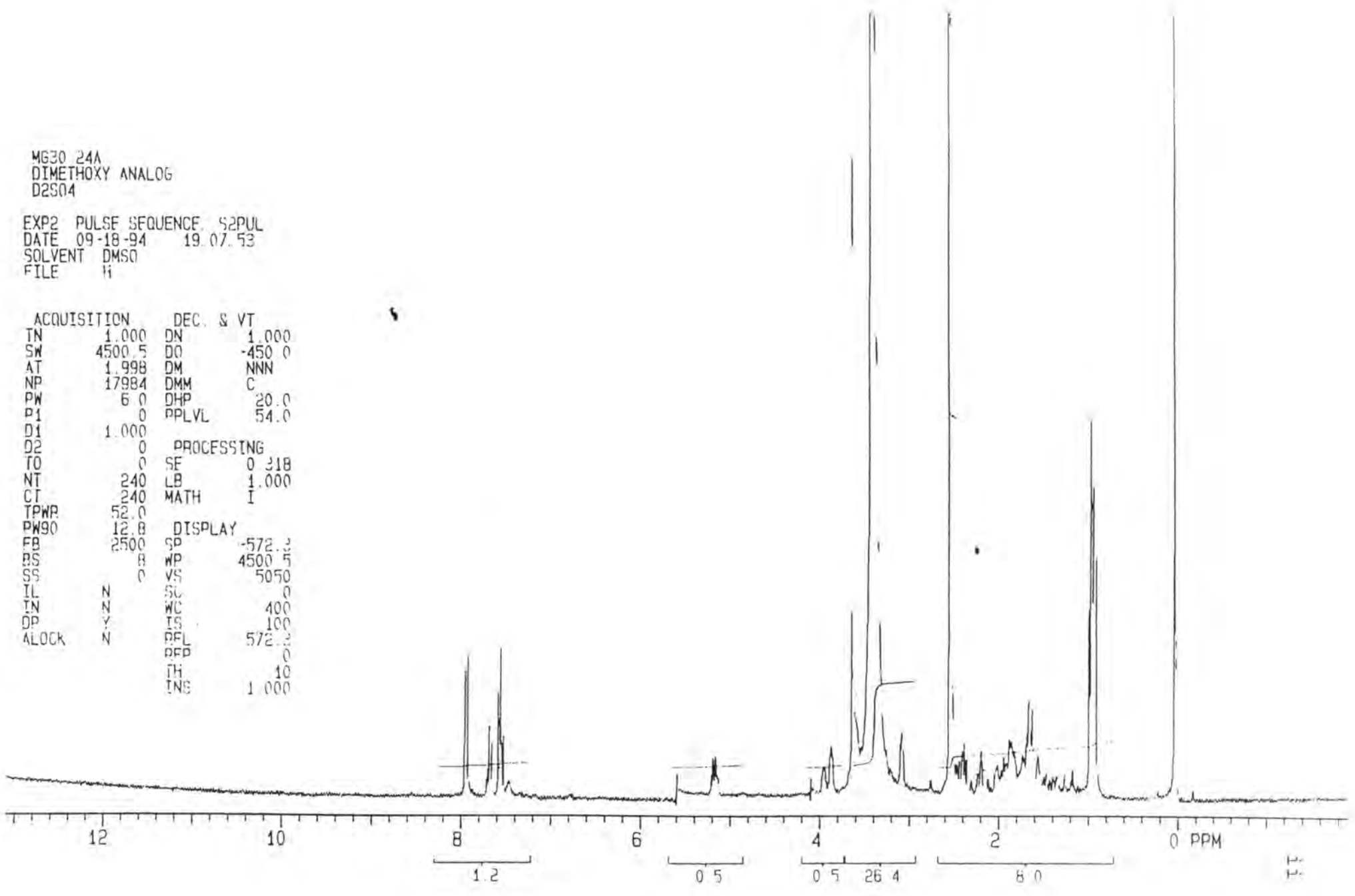
APPENDIX A

Representative Analytical Sprectra for the N-alkyl and N-acyl Norcocaine Derivatives



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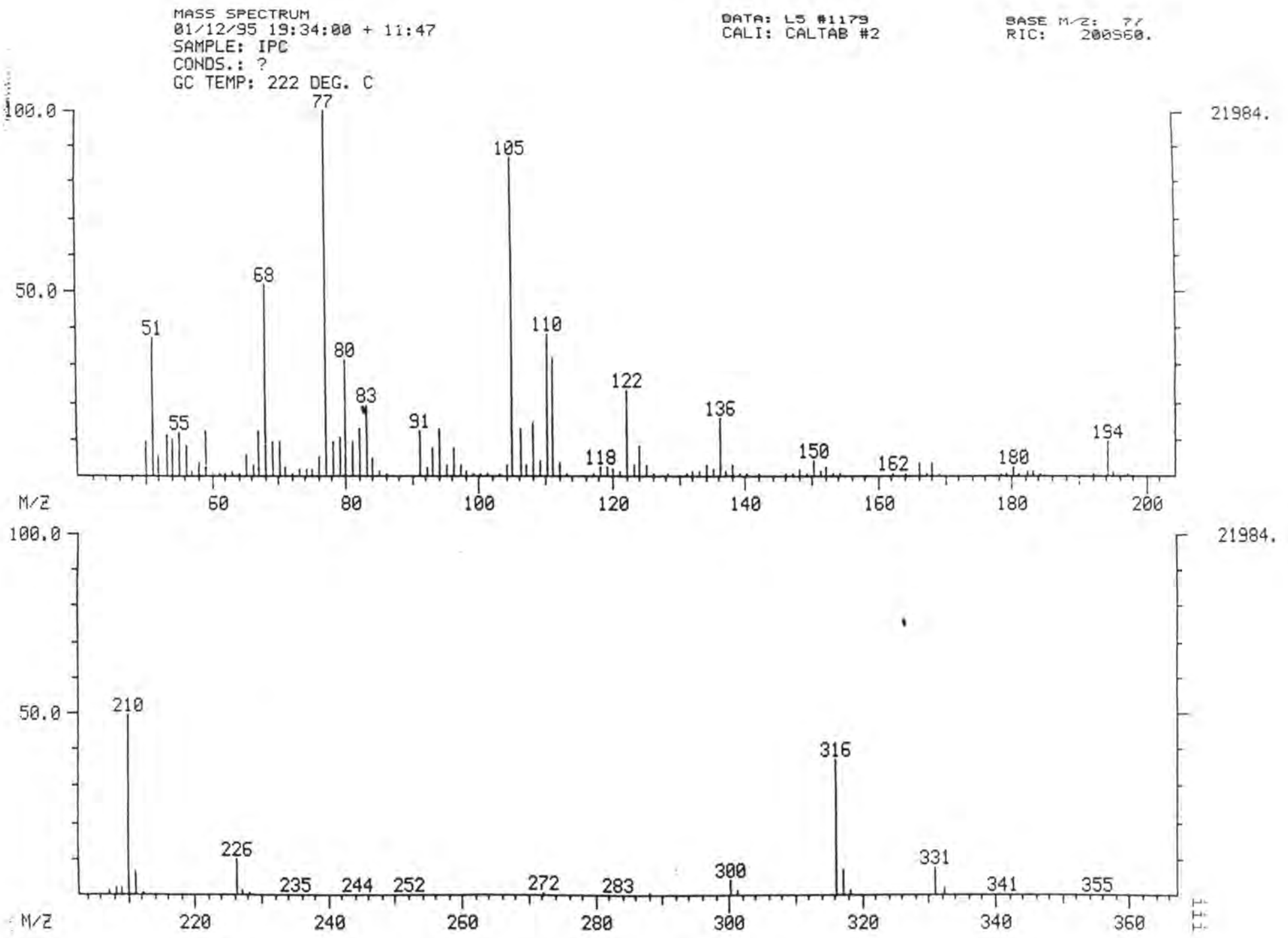
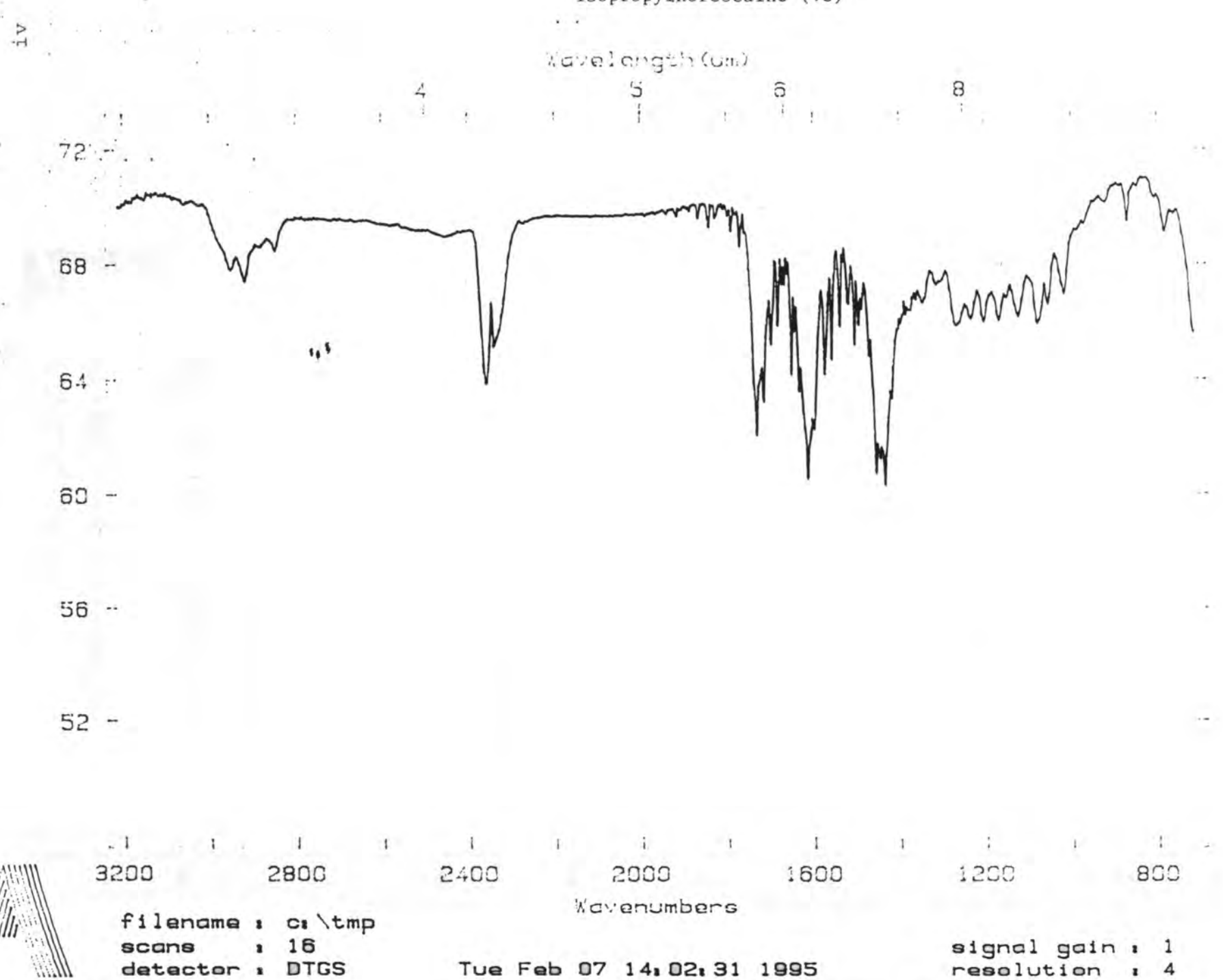


isopropylnorcocaine (Vc)

isopropylnorcocaine (Vc)



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Tue Feb 07 14:02:31 1995

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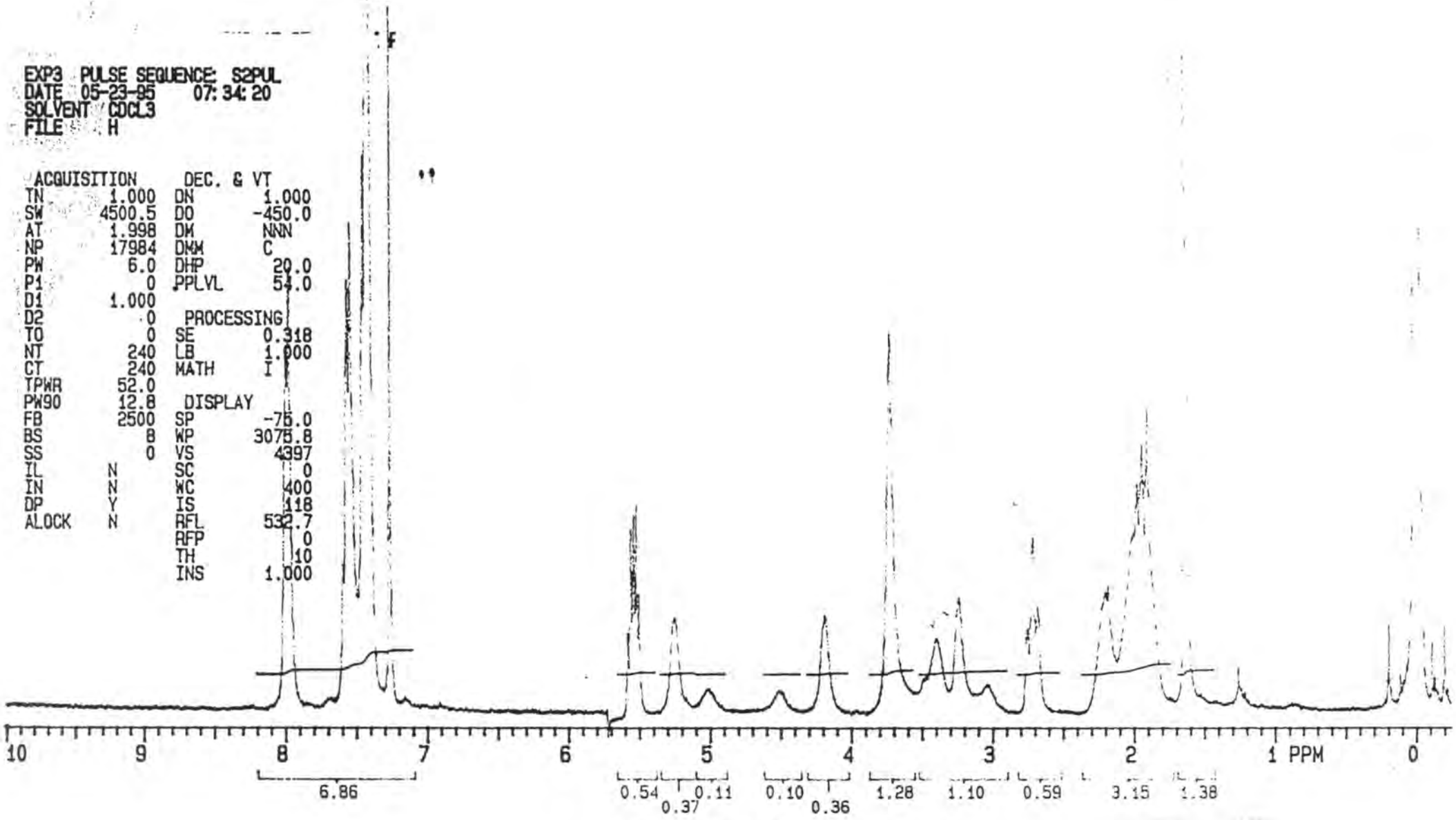

Benzoylnorcocaine (Ve)

02/13/95 18:54:00 + 11:02
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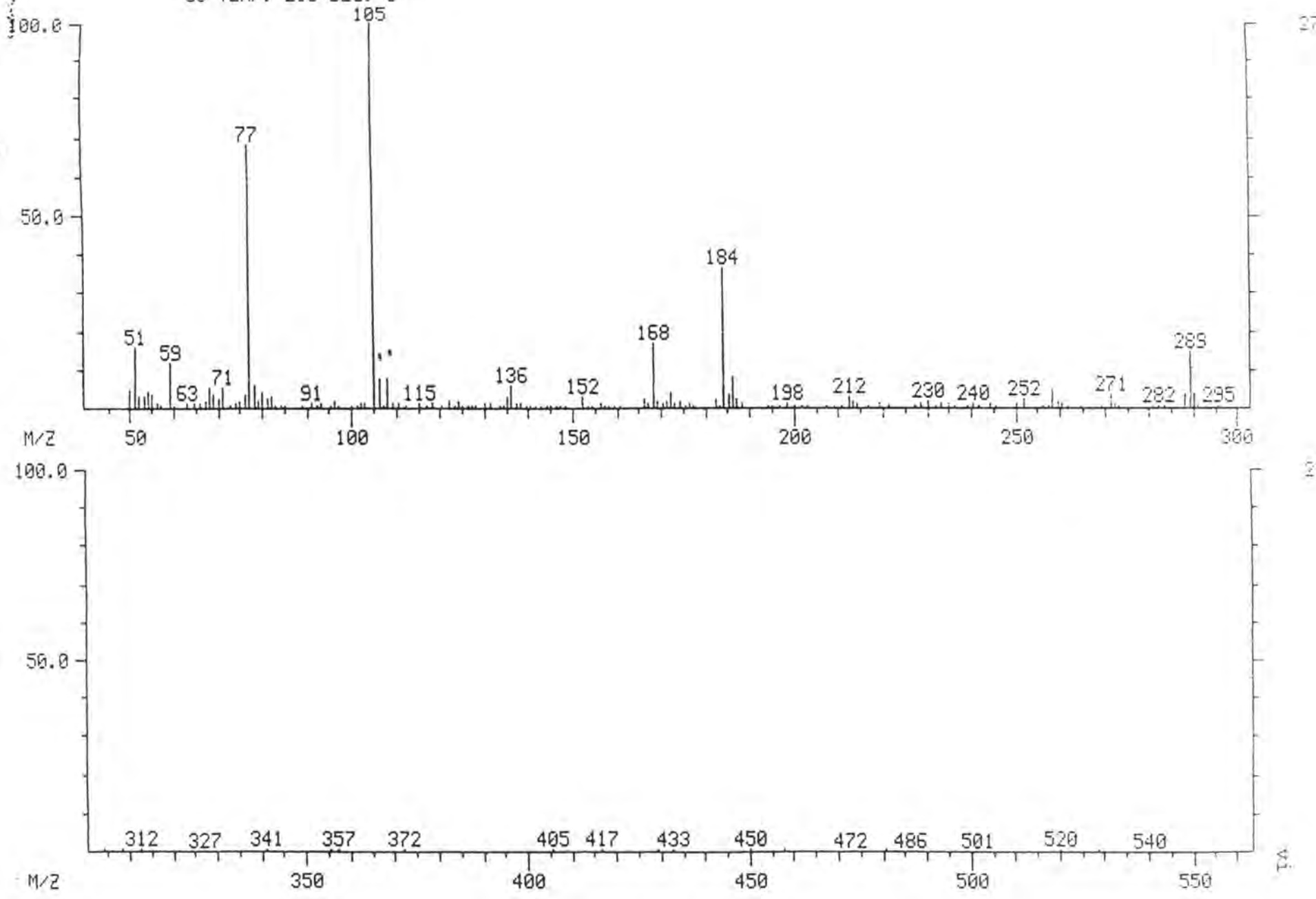
CONDS.: WORKUP
GC TEMP: 218 DEG. C

CALI: CALTAB #2

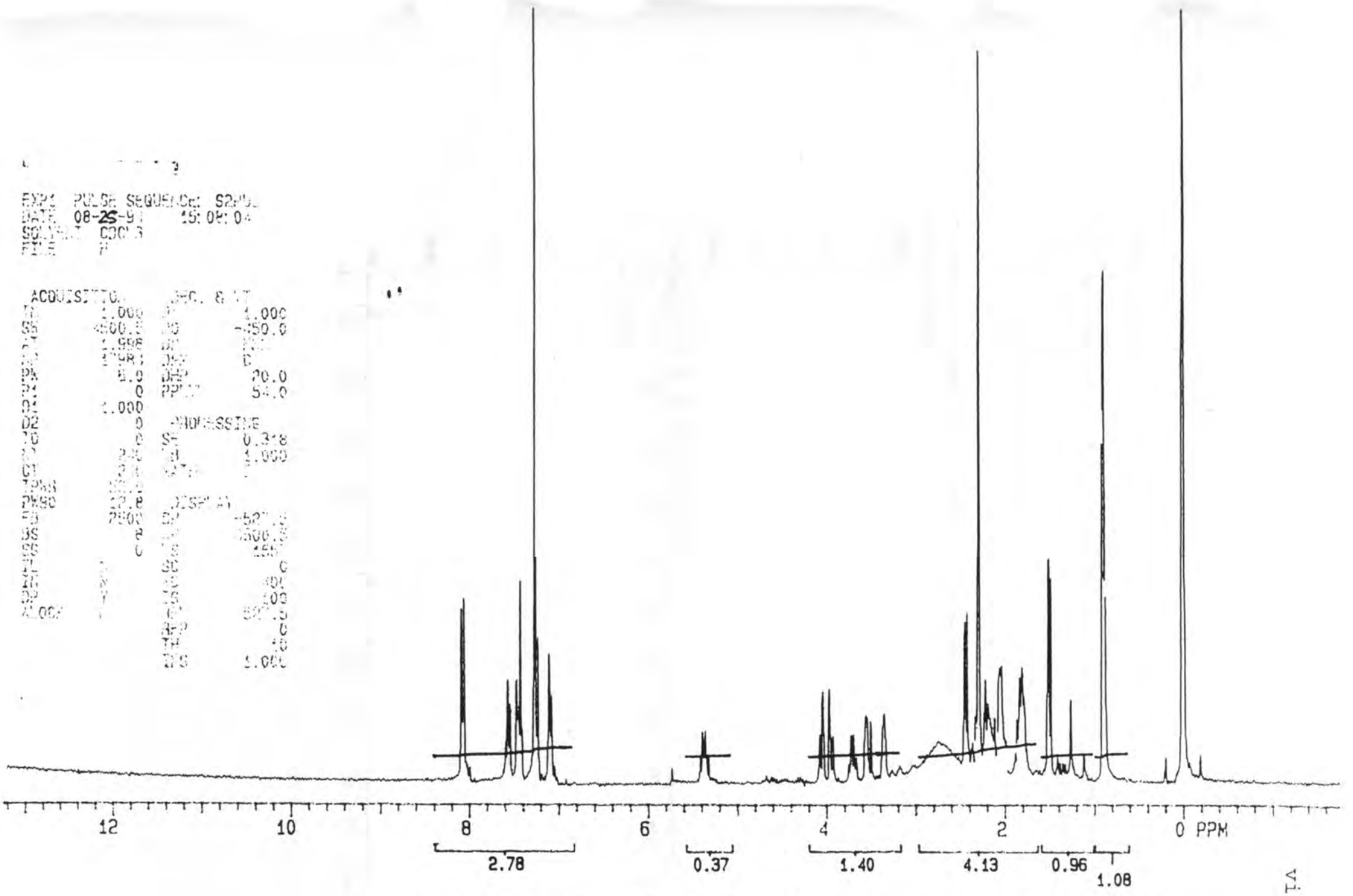
RIC: 13584.



Benzoylnorcocaine (Ve)

APPENDIX B

Representative Analytical Spectra for the Benzoylecgonine Derivatives



2-(4-isobutylphenyl)propionic acid, (3-benzoyloxy-2-tropyl)methyl ester

GEN\BARR2
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and consists of 811 scans acquired at a rate of 1.111 seconds
Solid probe
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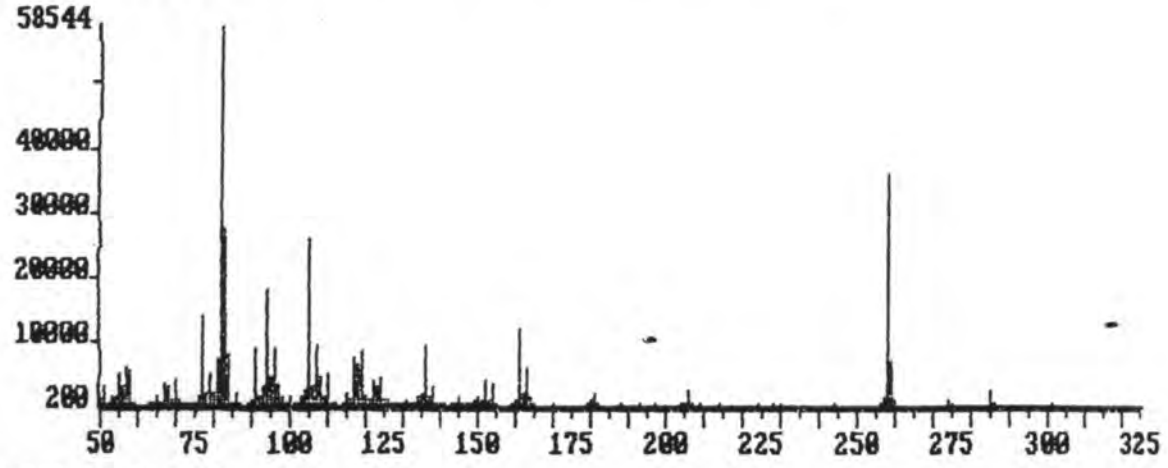
Masses were acquired as:
50- 600

Display X axis from 325 to 600 on marked plot

Scan at 37.0333



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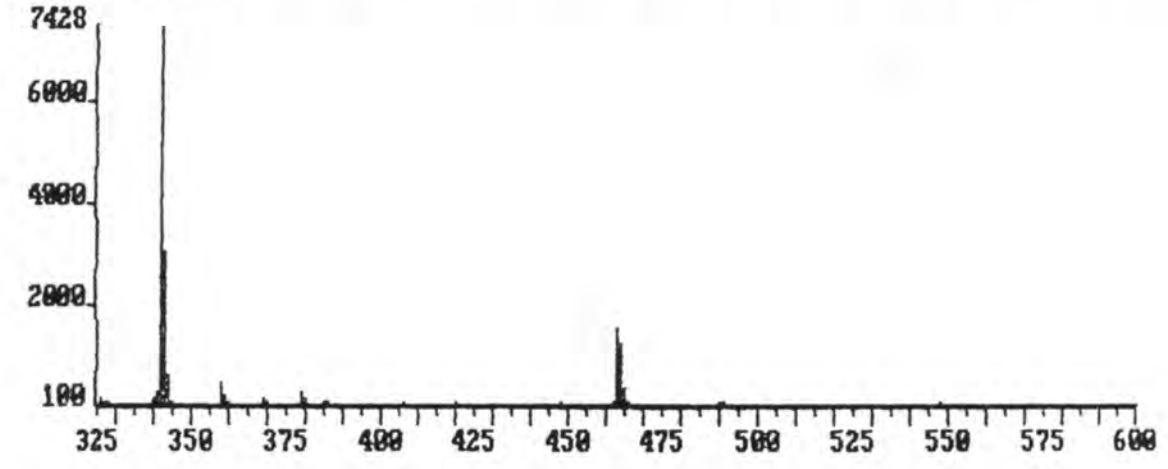

2-(4-isobutylphenyl)propionic acid, (3-benzoyloxy-2-tropylmethyl ester