The analytical procedure was employed for the determination of indoprofen levels in human milk from seven subjects (Table II). All samples were analyzed in duplicate, and each value is the average of these determinations. The relative standard deviation of pairs was 5.1%. Subjects 1, 3, and 4 had detectable levels of a component in the baseline samples, and these values were subtracted to obtain the corrected indoprofen levels. The impurity level was similar to that observed previously (~10 ng/ml). This interfering compound was possibly present in all samples but was not completely removed in some during the sample preparation. Thirteen recovery samples were analyzed (Table I, Part B). The average recovery was 94% with a standard deviation of ±8. Representative indoprofen chromatograms in milk samples are presented in Fig. 2. For the 20 spiked samples analyzed, the average recovery was 96%.

Plasma samples were obtained at 2.0 hr after administration for the single-dose subjects and at 2.0, 26, and 50 hr after the first dose for multiple-dose subjects. Each plasma sample was analyzed in duplicate, and the averages are given in Table II. The precision of the plasma determinations was excellent, with a relative standard deviation of pairs of 5.1%.

Indoprofen calibration standards were analyzed after every fourth sample to determine the relative weight response for drug level calculations. New standards were prepared for each subject. A comparison of the relative weight responses obtained during milk sample analysis is given in Table III. During the 15-day period, the day-to-day variation in standards was very small, with a relative standard deviation of 3.2% for the relative weight response. Thus, the analytical system had excellent stability and provided quantitative data for extended periods.

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Simple Analogs of the Toxin Callicarpone

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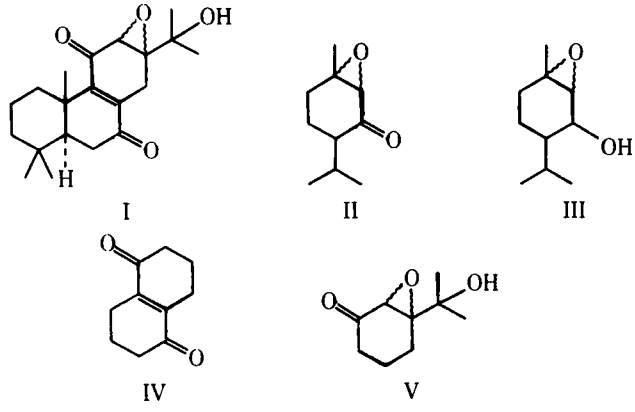
Abstract □ Callicarpone, a component 10 times as toxic to fish as rotenone, has been isolated from the leaves of *Callicarpa candicans*. It is reasonable to assume that callicarpone will act as an insecticidal agent as does rotenone. Therefore, the structure–activity relationship of callicarpone was examined by synthesizing a series of compounds having certain of its structural features. Those compounds were tested for insecticidal and antimicrobial activities. A study of synthetic analogs elucidated the functional group chemistry of callicarpone so that a synthesis might be undertaken. Piperitone oxide showed ~1/100th the activity of rotenone against *Daphnia magna*. 1-(α-Hydroxyisopropyl)-3-oxocyclohexene oxide showed activity against mycobacterium while 2,3,4,6,7,8-hexahydronaphthalene-1,4-dione showed inhibitory activity against the mycobacterium and two yeasts.

Keyphrases □ Callicarpone—analogs, structure–activity relationships, toxicity, antimicrobial activity □ Insecticides—callicarpone, analogs, structure–activity relationships, toxicity, antimicrobial activity □ Structure–activity relationships—callicarpone analogs, antimicrobial activity

The use of decoctions of various plants as fish poisons has been practiced for many centuries by native tribes in Africa, India, and South America. Usually, the poisonous plants were macerated with water, the decoction was poured into a selected body of water, and the fish that rose to the surface were collected. It was reasonable to assume that these poisonous plants should exhibit toxicity against organisms besides fish, and some of these plants have been found to contain useful insecticides.

A component toxic to fish has been isolated from the leaves of *Callicarpa candicans* (1). These leaves have long been used for stupefying fish by natives of Palau and the Philippine Islands. The active principle was named callicarpone (I). Callicarpone exhibited 10 times stronger toxicity against loach fish (*Misgurnus anguillicaudatus*) than did rotenone. The structure of callicarpone was deduced from spectral and chemical evidence.



Since callicarpone possesses stronger toxicity against fish than does rotenone, it is reasonable to assume that callicarpone also might act as an insecticidal agent. Therefore, a study of the structure–activity relationship of callicarpone was initiated by synthesizing a series of compounds having certain of its structural features and by testing these compounds for their toxicity to *Daphnia magna* (a fresh water crustacean) (2). To determine if these functional groups might show a more general biological activity, the agents also were tested for antimicrobial activity. A study of synthetic analogs provided information about the functional group chemistry of callicarpone so that a synthesis might be undertaken.

Callicarpone possesses some unique structural and

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functional features, especially in rings B and C. To determine the structure–activity relationship, these structural and functional features were incorporated into compounds II–V.

DISCUSSION

Compound II possessed the α-epoxyketone functionality with a comparable substitution pattern around the epoxide ring and the carbonyl group as found in callicarpone. To determine the importance of the α-epoxyketone for biological potency, III also was synthesized; it incorporated all of the features of II, except that the carbonyl group was replaced by a hydroxyl group. This compound also allowed study, independently, of the activity of an α-hydroxy epoxide function, a system present in the parent callicarpone.

Compound IV possessed the 1,4-enedione functionality incorporated into a hexahydronaphthalene moiety as found in callicarpone. The importance of an α-hydroxyisopropyl group associated with the α-epoxyketone in callicarpone could be tested by synthesis of V, which incorporated the α-hydroxyisopropyl group in a comparable position to the α-epoxyketone as found in callicarpone.

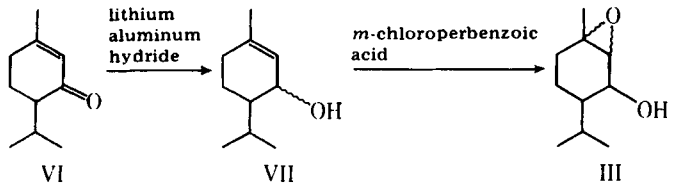
The synthesis of piperitone oxide II was accomplished by treating piperitone VI with hydrogen peroxide and base according to the organic synthesis procedure (3). The II yield was low and erratic with the use of 6 *N* NaOH. A substantial amount of diol was recovered from the reaction mixture. The yield was significantly improved (to >75%) when the strength of the base was lowered to 4 *N* NaOH. A pure sample of II, whose physical properties were consistent with those reported (4), was recovered from the reaction mixture by distillation under vacuum.

The synthesis of III was initiated by reducing VI with sodium borohydride in methanol at room temperature according to a literature procedure (5). However, the reaction failed to yield any appreciable amount of VII. Lithium aluminum hydride reduction of piperitone, according to a literature procedure (6), resulted in the isolation of the desired alcohol (VII) in a 95% yield. The synthesis of III was accomplished by subjecting VII to *m*-chloroperbenzoic acid buffered with sodium acetate in methylene chloride at 0–5° (7). The pure epoxyalcohol (III) was obtained in a 70% yield from the reaction product by distillation under vacuum (Scheme I).

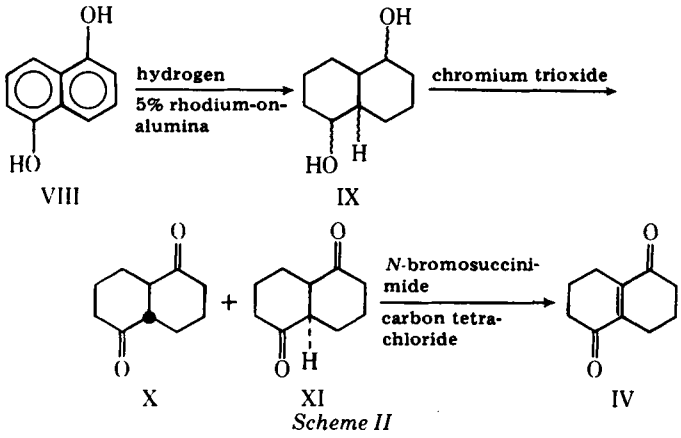
Campbell and Harris (8) reported the synthesis of 2,3,4,6,7,8-hexahydronaphthalene-1,5-dione (IV) in a <0.5% overall yield from Δ^{9-10} octalin. Alternatively, Johnson *et al.* (9) reported the synthesis of decalin-1,5-diones (X and XI) from naphthalene-1,5-diol (VIII) by reduction with Raney nickel at high temperature and pressure and subsequent oxidation. Since rhodium and ruthenium catalysts were unknown at the time of that work, an attempt was made to reduce the naphthalene-1,5-diol with 5% rhodium on alumina at 60 psi (10), which resulted in the isolation of the desired decalin-1,5-diols (IX) in a >90% yield (Scheme II).

The stereochemistry of the diols (IX) was not crucial for further transformations, so the mixture of stereoisomers was not separated at this juncture. The diols (IX) were subjected to modified Jones oxidation using chromium trioxide, benzene, sulfuric acid, and acetic acid at 0–5°. The *cis*- and *trans*-decalin-1,5-diones (X and XI) were obtained in a 2:3 ratio in an ~85% yield. These two compounds were separated by repeated fractional crystallization using benzene and petroleum ether (bp 60–65°). The physical properties of X and XI were consistent with those reported previously (9).

The mixture of *cis*- and *trans*-decalin-1,5-diones was refluxed with *N*-bromosuccinimide in carbon tetrachloride with irradiation with IR light (11). However, the anticipated monobromodecalin-1,5-dione was not obtained in a good yield. To improve the yield, the reflux time was increased to 48 hr. The isolated yellowish-brown solid, obtained in a 65% yield, was devoid of bromine. On repeated crystallization with petroleum ether (bp 60–65°), a light-yellow solid was obtained; it was characterized as IV on the basis of physical and spectral properties. Compound IV also could be obtained from the reaction product mixture by eluting it through



Scheme I



Scheme II

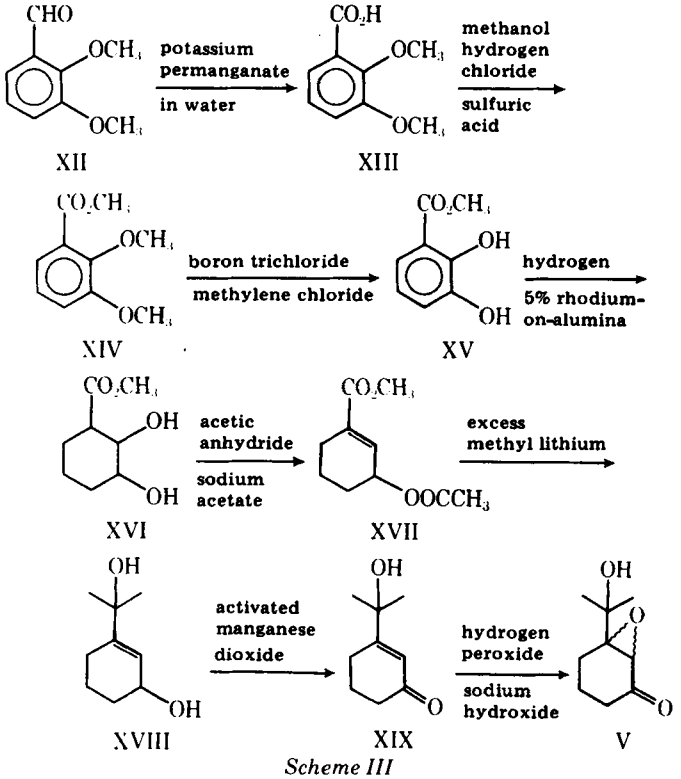
a short alumina column with cyclohexane or petroleum ether (bp 60–65°).

The synthesis of 1-(α-hydroxyisopropyl)-3-oxocyclohexene oxide (V) (Scheme III) was initiated from commercially available 2,3-dimethoxybenzaldehyde (XII), which was oxidized with aqueous potassium permanganate at steam bath temperature by an organic synthesis procedure (12) to 2,3-dimethoxybenzoic acid (XIII) in a >90% yield. The acid XIII was esterified by treatment with methanolic hydrogen chloride containing a trace of sulfuric acid prior to the cleavage of the methyl ethers (13).

Gero (14) used boron trichloride to cleave methyl ethers in the presence of an ester group. Application of this procedure to XIV, using a fourfold excess of boron trichloride in methylene chloride at −78°, gave the desired catechol (XV) in an 85–90% yield.

The synthesis of methyl-2,3-dihydroxycyclohexane carboxylate (XVI) was accomplished by the reduction of XV over 5% rhodium on alumina at 3 atmospheres of hydrogen pressure. Sodium methoxide treatment of XVI failed to provide the desired elimination product, methyl-3-hydroxycyclohex-1-ene carboxylate. A yellowish-brown solid obtained from the reaction could not be identified. Acetylation of XVI, using acetic anhydride in pyridine (XV), failed to give appreciable diacetate.

The synthesis of XVII was achieved by refluxing XVI with acetic anhydride in the presence of sodium acetate for 72 hr. A mixture of XVII and the diacetate of XVI was obtained in an ~70–75% yield. The two components were separated by chromatography on an alumina column. The formation of XVII could be explained by sodium acetate-catalyzed elimination of acetic acid from the diacetate. This hypothesis is supported



Scheme III

by the observation that the diacetate was transformed to XVII under these reaction conditions. Treatment of XVII with six equivalents of methyl lithium in ether at room temperature for 16 hr (15) produced XVIII in a good yield. Conversion of XVIII to XIX was performed in a 60% yield by treating XVIII with commercially available activated magnesium dioxide in hexane at room temperature for 96 hr (16).

The final step in the synthesis of V was achieved in a 65% yield by treating XIX with hydrogen peroxide and 4 *N* NaOH. The structure of V was established on the basis of spectral data. The IR spectrum showed an intense band at 1700 cm^{-1}, indicating the presence of a saturated six-membered alicyclic ketone. The PMR spectrum of V indicated the presence of the α-hydroxyisopropyl group at δ 1.4 ppm integrating for six protons. This signal was shifted downfield on the addition of trichloroacetyl isocyanate. The PMR spectrum also showed a sharp singlet integrating for one proton at δ 3.4 ppm, supporting the presence of the epoxide ring. The mass spectrum contained peaks at *m/e* 170 (M^+), 152, 137, 124, 83, and 82.

BIOLOGICAL RESULTS

Compounds II–V were assayed for antimicrobial activity[1] using the standard agar well-diffusion technique. Organisms included in the assay were *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Bacillus subtilis* (ATCC 6633), *Mycobacterium smegmatis* (ATCC 14468), *Aspergillus niger* (ATCC 11394), *Saccharomyces cereviseae* (ATCC 9763), and *Candida albicans* (ATCC 10231).

Stock cultures (except *A. niger*) were inoculated into 50 ml of culture broth (Eugon broth for bacteria and Mycophil broth for the yeasts) and incubated for 24 hr with shaking. The 24-hr cultures were diluted 1:50 in sterile distilled water, and agar plates (Eugon or Mycophil) were streaked (quadrant method) with the 1:50 dilution using a sterile dacron swab. For *A. niger*, a sterile water suspension of conidia was streaked on Mycophil agar.

Cylindrical plugs were removed from the agar plates using a sterile cork borer to produce wells having a diameter of 11 mm. Each 100 × 15-mm plate had two wells, one for the test solution and one for the solvent blank. With a micropipet, 100 μl of the test solution or solvent was added to each well. Compounds were tested routinely at a 2-mg/ml concentration in ethanol. A standard antibiotic for each organism was incorporated into the screen. Streptomycin sulfate (2 mg/ml) was used for the bacteria, and amphotericin B (250 μg/ml) was used for the fungi and yeasts. The results were read after 24 and 48 hr and are reported as the average radius of the inhibition zone measured in millimeters from the edge of the well to the edge of the inhibition zone.

Compounds II and III showed no inhibition of any organism. Compound IV showed slight inhibition of the two yeasts and the mycobacterium (Table I). Compound IV might distantly resemble the quinone compounds, which are so widely prevalent in biological systems, although it is premature to draw any conclusion regarding its mechanism of action. Compound V showed good inhibition (17 mm) of the mycobacterium but no inhibition of any other organism.

Compounds II–IV also were tested against *D. magna* (a fresh water organism). Solutions containing 80, 8, 0.8, or 0.08 mg of II, III, or IV/liter were tested against *D. magna* using a rotenone standard (2). Toxicity to the *Daphnia* from these compounds was observed every 30 min. Compound II was the most active of the compounds tested. It possessed ~1/100th the activity of rotenone. Compound III was devoid of any activity, while Compound IV was ~1/1000th as active as rotenone.

EXPERIMENTAL[2]

Piperitone Oxide (II)—In a 250-ml three-necked flask equipped with a mechanical stirrer, a dropping funnel, and a thermometer was placed a solution of 7.1 g (0.046 mole) of piperitone (VI) and 15 ml (0.12 mole) of 30% H_2O_2 in 50 ml of methanol. After the contents of the flask had been cooled to 15° by an ice–water bath, 15 ml (0.06 mole) of 4 *N* NaOH was added dropwise over 1 hr. During the addition, the temperature of the reaction mixture was maintained at 15–20° with the ice–water bath. After the addition was complete, the resulting mixture was stirred for 3 hr at room temperature.

The reaction mixture was poured into 100 ml of water and extracted with three 100-ml portions of ether. The ethereal extracts were combined, washed with water, dried over magnesium sulfate, concentrated, and distilled *in vacuo* to give 5.3 g (70%) of II, bp 63–65° (1 mm) [lit. (4) bp 66–67° (1 mm)]; IR (liquid film): 1705 (C=O) and 780 and 840 (epoxide) cm^{-1}; PMR ($CDCl_3$): 3.1 (s, 1, epoxide), 0.8–1.0 (dd, 6, CH_3CHCH_3), and 1.4 (s, 3, CH_3CCH); mass spectrum: 168 (M^+, 2% of base peak), 126 ($M^+ - C_3H_6$, 20% of base peak), 140 (M^+ – C=O, 5% of base peak), and 56 (base peak, 100%).

1-Methyl-4-isopropyl-Δ^1-cyclohexene-3-ol (VII)—In a 250-ml three-necked flask equipped with a magnetic stirrer, a dropping funnel, and a condenser was placed a solution of 8.0 g (0.053 mole) of VI in 100 ml of ether. Then a solution of 0.80 g (0.021 mole) of lithium aluminum hydride in 50 ml of ether was added during 30 min. The mixture was refluxed for 0.5 hr. Excess lithium aluminum hydride was decomposed with 5 ml of 50% ethanol followed by 10 ml of water until the precipitation of aluminum oxide had stopped. The ether layer was separated, washed with water, dried over magnesium sulfate, concentrated, and distilled to give 7.8 g (95%) of VII; IR (liquid film): 3400 (OH), 1640 (C=C), and 1380 (C—CH_3) cm^{-1}; PMR ($CDCl_3$): 5.5 (bs, 1, C=CH), 4.1 (m, 1, CHOH), and 1.8 (s, 3, vinylic methyl).

1-Methyl-3-hydroxy-4-isopropylcyclohexene Oxide (III)—In a 250-ml three-necked flask equipped with a stirrer, a dropping funnel, and a thermometer was placed a solution of 4.5 g (0.03 mole) of VII in 100 ml of methylene chloride. The solution was cooled to 20° by an ice–water bath. A solution of 9.5 g (0.045 mole) of 85% *m*-chloroperbenzoic acid, containing 1.5 g of sodium acetate in 50 ml of methylene chloride, was added dropwise in 15 min. The resulting mixture was stirred at room temperature for 20 hr. The contents of the flask were poured into 150 ml of water, the organic layer separated, and the aqueous layer was extracted with two 50-ml portions of methylene chloride.

The combined methylene chloride layers were washed with two 25-ml portions of 10% sodium carbonate and two 50-ml portions of water. The organic layer was dried over magnesium sulfate, concentrated, and distilled *in vacuo* to give 3.5 g (70%) of III; IR (liquid film): 3450 (hydroxyl), 1380 (C—CH_3), and 840 and 770 (epoxide) cm^{-1}; PMR ($CDCl_3$): 3.9 (m, 1, CHOH), 3.3 (bs, 1, C—CH), and 1.2 (s, 3, epoxide methyl); mass spectrum: 170 (M^+, 5% of base peak), 152 ($M^+ - H_2O$, 7% of base peak), 85 (15% of base peak), 84 (base peak, 100%), and 56 (30% of base peak).

Decalin-1,5-diol (IX)—A solution of 20 g of 1,5-dihydroxynaphthalene (VIII) in 500 ml of 95% ethanol was boiled with 4 g of activated charcoal for 15 min and filtered while hot. The filtrate was again boiled with 4 g of activated charcoal for 5 min and filtered while hot. Upon cooling to ice bath temperature, a first crop of VIII crystallized. The crystals were separated by filtration, and the filtrate was concentrated on the rotary evaporator, filtered, and chilled in the refrigerator. A second crop of VIII was obtained, yielding a total recovery of 11.5 g.

A solution of 10.0 g (0.063 mole) of VIII in 100 ml of methanol was hydrogenated in the presence of 5 g of 5% rhodium-on-alumina at an initial pressure of 65 psi. This sample was left until no additional fall in pressure was observed (usually 48 hr). The catalyst was removed by filtration, and the solvent was removed on the rotary evaporator. The residue was treated with 20 ml of 10% NaOH and 50 ml of benzene and shaken vigorously. A white solid material was separated, filtered, and washed twice with water and then with ether. A yield of 9.1 g (86%) of IX

Table I—Inhibition of Microorganisms by IV

Organism	Zone of Inhibition, mm
Yeasts and fungi	
A. niger	0
C. albicans	4
S. cereviseae	3
Mycobacteria	
M. smegmatis	2
Bacteria	
S. aureus	0
E. coli	0
P. aeruginosa	0
B. subtilis	0

[1] Department of Pharmacognosy, University of Mississippi, University, MS 39677.

[2] All melting points were taken on a Thomas-Hoover Unimelt apparatus and are corrected. Analyses were performed either by Midwest Microlab, Inc., Indianapolis, Ind., or on an F and M model 185 C, H, N analyzer at the University of Kansas. IR data were recorded on Beckman IR-8, IR-10, and IR-33 spectrophotometers. PMR data were recorded on Varian A-60, A-60A, and T-60 analytical spectrometers with tetramethylsilane as the internal standard. PMR data are reported as δ values (parts per million). Mass spectra were recorded on a Varian Atlas CH 5 at 70 ev at a resolution of ~4000.

was obtained, mp 208–209° [lit. (12) mp 210–211°]; IR (KBr disk): 3400 (hydroxyl), 1450 (methylene), and 1030–1050 (COH) cm^{-1}.

Decalin-1,5-diones (X and XI)—A suspension of 5.0 g (0.03 mole) of IX in 75 ml of benzene was cooled to 0–5° by cooling in an ice–water bath. To this suspension was added dropwise, with stirring, a cold solution of 7 g of sodium dichromate in 5.25 ml of glacial acetic acid, 9.5 ml of concentrated sulfuric acid, and 50 ml of water. During the addition, which required ~2 hr, the temperature of the reaction mixture was not allowed to exceed 5°.

After the addition was complete, the mixture was stirred for an additional 3 hr at 0–5° and then allowed to stand at room temperature overnight. The aqueous layer was separated and extracted with two 25-ml portions of benzene. The combined benzene layers were washed with water, with saturated sodium bicarbonate, and finally with water. The benzene was removed on the rotary evaporator to give 4.1 g (83%) of the *cis*- and *trans*-decalin-1,5-diones (X and XI). These compounds were separated from the mixture by fractional crystallization using benzene and petroleum ether (bp 60–65°), mp (X) 80–81° [lit. (12) mp 79–80°] and mp (XI) 165–166° [lit. (12) mp 166–167°]; IR (KBr disk): 1700 (C=O) and 1460 (methylene) cm^{-1}.

2,3,4,6,7,8-Hexahydronaphthalene-1,5-dione (IV)—A solution of 1.4 g (0.009 mole) of X and XI and 1.6 g (0.01 mole) of *N*-bromosuccinimide, recrystallized from water, in 50 ml of carbon tetrachloride was refluxed for 48 hr. The solution was irradiated intermittently with IR light. The floating succinimide residue was filtered and washed with carbon tetrachloride. The organic layer was concentrated to give 0.95 g (65%) of a yellow solid which, on recrystallization from petroleum ether, gave a light-yellow solid (IV), mp 110–111° [lit. (10) mp 112–113°]; IR (KBr disk): 2960 (CH), 1710, 1675 (C=O), and 1640 (C=C) cm^{-1}; PMR ($CDCl_3$): 2.5 (m, 6, allylic and methylenes alpha to carbonyl), and 2.0 (m, 4, methylene); mass spectrum: 164 (M^+, 40% of base peak), 136 (M^+ – C=O, 70% of base peak), and 84 (base peak, 100%).

2,3-Dimethoxybenzoic Acid (XIII)—A mixture of 2,3-dimethoxybenzaldehyde (XII) (100 g, 0.6 mole) and 1 liter of water was placed in a three-necked flask equipped with a mechanical stirrer, a dropping funnel, and a thermometer. The flask was placed on a steam bath, heated to 70–80°, and stirred. A solution of 150 g of $KMnO_4$ in 800 ml of water was allowed to flow into the emulsion of XII over 40–45 min. The stirring was continued for 90 min.

Sufficient 10% potassium hydroxide solution was added to make the reaction mixture alkaline, the mixture was filtered while hot, and the manganese dioxide was washed with two 200-ml portions of hot water. The filtrate was acidified with concentrated hydrochloric acid until no further precipitate formed. The resulting XIII was filtered, washed with cold water, and dried. The yield was 95 g (92%), mp 121° [lit. (17) mp 120–122°].

Methyl-2,3-dimethoxybenzoate (XIV)—A solution of XIII (90 g, 0.53 mole) in 500 ml of methanol saturated with dry hydrogen chloride gas was prepared. One milliliter of concentrated sulfuric acid was added, and the mixture was refluxed for 12 hr. The reaction mixture was concentrated on the rotary evaporator, the residue was extracted with ether, and the ethereal layer was washed with water, saturated sodium bicarbonate solution, and then water. It was then dried over magnesium sulfate and concentrated to give 81.5 g (85%) of XIV, mp 58–59° [lit. (18) mp 57–58°].

Methyl-2,3-dihydroxybenzoate (XV)—In a 1-liter three-necked flask equipped with a mechanical stirrer was placed a solution of 55 g (0.27 mole) of XIV in 400 ml of methylene chloride. Boron trichloride (90 g, 0.81 mole) was condensed into the solution at −78°. The reaction mixture was stirred at −78° for 90 min and then allowed to stir at room temperature overnight. The resulting boron trichloride complex was decomposed with careful addition of water, the organic layer separated, and the aqueous layer was extracted with two 100-ml portions of methylene chloride.

The methylene chloride layers were combined and washed with three 100-ml portions of water, saturated sodium bicarbonate solution, and then water. Then they were dried over magnesium sulfate and concentrated to yield 45 g (85%) of XV, mp 78–80°; PMR ($CDCl_3$): 7.0 (m, 3, aromatic), 5.9 (bs, 1, OH), 10.8 (s, 1, OH), and 3.9 (s, 3, $COOCH_3$).

Methyl-2,3-dihydroxycyclohexane Carboxylate (XVI)—A solution of 20.0 g (0.12 mole) of XV and methanol (200 ml) was hydrogenated in the presence of 5 g of 5% rhodium-on-alumina at an initial pressure of 60 psi for 24 hr. The catalyst was removed by filtration, and the solvent was removed on a rotary evaporator to give 19.8 g (96%) of an oil (XVI); IR (liquid film): 3450 (OH), 2960 (CH), and 1735 ($COOCH_3$) cm^{-1}; PMR ($CDCl_3$): 4.3 (m, 1, *H*—C—OH), 4.1 (m, 1, *H*—C—OH), 3.8 (s, 3, $COOCH_3$), and 1.6 (m, 8, methylene).

Methyl-3-acetoxy-Δ^1-cyclohexene Carboxylate (XVII)—A solution of 20.0 g (0.115 mole) of XVI in 250 ml of acetic anhydride, containing 6.5 g of anhydrous sodium acetate, was refluxed for 72 hr. The solution was filtered, excess acetic anhydride was distilled *in vacuo*, and the residue was poured into 150 ml of 10% sodium carbonate solution and kept overnight. The solution was extracted with three 100-ml portions of ether, and the ether layers were combined and washed with water, dried over magnesium sulfate, and concentrated to give 15.5 g of an oil containing a mixture of XVII and methyl-2,4-diacetoxycyclohexane carboxylate.

The crude mixture was adsorbed onto a neutral alumina column (activity grade II) and eluted with 20% ethyl acetate in cyclohexane. A yield of 8.5 g (35%) of pure XVII was eluted from the column; IR (liquid film): 2960 (CH), 1730 ($COOCH_3$ and acetate), 1650 (C=C), and 1370 ($C—CH_3$) cm^{-1}; PMR ($CDCl_3$): 6.8 (m, 1, C=CH), 5.4 (m, 1, $CHOOCH_3$), 3.8 (s, 3, $COOCH_3$), and 2.1 (s, 3, acetate).

1-(α-Hydroxyisopropyl)-Δ^1-cyclohexane-3-ol (XVIII)—In a 250-ml three-necked flask equipped with a stirrer and a dropping funnel was placed a solution of 4.0 g (0.02 mole) of XVII in 50 ml of ether under an atmosphere of argon gas. The solution was cooled to 0–5° by an ice bath. A 120-ml aliquot of a 1 *M* solution (0.12 mole) of methyl lithium in ether was added dropwise over 1 hr. The resulting solution was allowed to stir overnight at room temperature. The solution was poured into 100 ml of 5% ammonium bicarbonate, the ethereal layer separated, and the aqueous layer was extracted with two 50-ml portions of ether.

The combined ether layers were washed with water, dried over sodium sulfate, and concentrated to give 2.6 g (83%) of XVIII; IR (liquid film): 3400 (OH), 3000 (CH), 1660 (C=C), and 1370 and 1380 ($CH_3—C—CH_3$) cm^{-1}; PMR ($CDCl_3$): 5.8 (m, 1, C=CH), 3.9 (m, 1, *CH*—OH), and 1.32 [s, 6, $CH_3—C(—OH)—CH_3$].

1-(α-Hydroxyisopropyl)-Δ^1-cyclohexane-3-one (XIX)—A solution of 1.5 g (0.0098 mole) of XVIII in 50 ml of hexane, containing 5 g of activated manganese dioxide, was allowed to stir at room temperature for 96 hr. The solution was filtered, and the solvent was removed *in vacuo* to give 1.05 g (65%) of XIX; IR (liquid film): 3450 (OH), 1675 (C=O), and 1620 (C=C) cm^{-1}; PMR ($CDCl_3$): 6.15 (s, 1, C=C—H), 2.4 (m, 2, CH_2C=O), and 1.4 [s, 6, $CH_3—C(—OH)—CH_3$]; mass spectrum: 154 (M^+, 1% of base peak), 136 ($M^+ - H_2O$, 2% of base peak), 139 ($M^+ - CH_3$, 2% of base peak), and 83 (base peak, 100%).

1-(α-Hydroxyisopropyl)-3-oxocyclohexane Oxide (V)—In a 100-ml three-necked flask equipped with a mechanical stirrer and a dropping funnel was placed a solution of 0.77 g (0.002 mole) of XIX and 1.5 ml (0.012 mole) of 30% H_2O_2 in 40 ml of methanol. After the contents of the flask were cooled to 15° by an ice–water bath, 1 ml (0.006 mole) of 4 *N* NaOH was added. The resulting mixture was stirred at room temperature for 4 hr and poured into 50 ml of water. Then the aqueous layer was extracted with three 30-ml portions of ether.

The ethereal layers were combined, washed with water, dried over magnesium sulfate, and concentrated *in vacuo* to give 0.55 g (70%) of V; IR (liquid film): 3400 (OH), 1705 (C=O), and 825 and 780 (epoxide) cm^{-1}; PMR ($CDCl_3$): 3.4 (s, 1, C—CH), 2.2 (t, 2, $CH_2—C$=O), and 1.4 (s, 6, $CH_3—C—CH_3$); mass spectrum: 170 (M^+, 1% of base peak), 152 ($M^+ - H_2O$, 2% of base peak), 83 (base peak, 100%), and 82 (8% of base peak).

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Kinetic Parameter Estimation by Numerical Algorithms and Multiple Linear Regression: Application to Pharmacokinetics

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Abstract □ Two numerical examples are presented to illustrate the application of the proposed method of parameter estimation in pharmacokinetics. Results for a system exemplifying first-order kinetics indicate that parameters estimated by the proposed procedure compare favorably with those estimated by a nonlinear regression method. In a simulated example characterized by Michaelis–Menten elimination kinetics, the accuracy of the estimated parameters was comparable to that expected, verifying the validity of the method. The importance of the numerical approximation algorithms was demonstrated also.

Keyphrases □ Pharmacokinetics—estimation by numerical algorithms and multiple linear regression □ Numerical algorithms—estimation of kinetic parameters □ Multiple linear regression—estimation of kinetic parameters □ Statistics—numerical algorithms, multiple linear regression, estimation of kinetic parameters

A general approach suitable for parameter estimation was reported previously (1). The strategy is to obtain equivalent mathematical expressions in linear form. Briefly, the procedure involves data transformation, usually by numerical integration and/or differentiation, followed by multiple linear regression. The application of this technique in pharmacokinetics is illustrated in the present report.

ESTIMATION PROCEDURE

Linear Case—Many pharmacokinetic processes are linear, and their description can be approximated by multiexponential equations. In a two-compartment open model (2, 3), the observed drug concentration in the central compartment, C_i, after intravenous administration can be described by:

$$C_i = a\exp(-\alpha t_i) + b\exp(-\beta t_i) \qquad i = 1, 2, \ldots, n \qquad \text{(Eq. 1)}$$

where a, b, α, and β are unknown constants and n is the number of observations[1]. If the elimination is assumed to take place from the central compartment only, as depicted in Scheme I, the relationships between the four constants and the model parameters become:

$$\alpha = 0.5[(k_{12} + k_{21} + k_{10}) + \sqrt{(k_{12} + k_{21} + k_{10}) - 4k_{21}k_{10}}] \qquad \text{(Eq. 2)}$$

$$\beta = 0.5[(k_{12} + k_{21} + k_{10}) - \sqrt{(k_{12} + k_{21} + k_{10}) - 4k_{21}k_{10}}] \qquad \text{(Eq. 3)}$$

$$a = D(\alpha - k_{21})/V(\alpha - \beta) \qquad \text{(Eq. 4)}$$

[1] For simplicity, subscript i is omitted and implied in most of the equations.

$$T \underset{K_{21}}{\overset{K_{12}}{\rightleftharpoons}} B \xrightarrow{K_{10}} U$$

Scheme I—Linear two-compartment open model depicting the body as composed of the central compartment B *(including blood) and the peripheral compartment* T, *where* k_{12} *and* k_{21} *are first-order intercompartmental transfer rate constants,* k_{10} *is the first-order elimination rate constant, and* U *is the eliminating compartment. The drug dose* D *is introduced into the central compartment at zero time. The drug concentration in the central compartment is defined as* C = B/V, *where* V *is the volume of distribution of the central compartment.*

$$b = D(k_{21} - \beta)/V(\alpha - \beta) \qquad \text{(Eq. 5)}$$

$$V = D/(a + b) \qquad \text{(Eq. 6)}$$

By applying the procedure described earlier (1) and recognizing that concentration at time zero is not an observation for a given experiment, the biexponential equation can be transformed into the following linear expression, which contains four terms:

$$C = \sum_{j=1}^{4} A_j X_j \qquad \text{(Eq. 7)}$$

where:

$$A_1 = \frac{D}{V} + \frac{K_{21}D}{V}t_1 - (\alpha + \beta)\int_0^{t_1} C\,dt - (\alpha\beta)\int_0^{t_1}\int_0^{t_1} C\,dt^2 \qquad \text{(Eq. 8)}$$

$$A_2 = \frac{K_{21}D}{V} - (\alpha\beta)\int_0^{t_1} C\,dt \qquad \text{(Eq. 9)}$$

$$A_3 = -(\alpha + \beta) \qquad \text{(Eq. 10)}$$

$$A_4 = -(\alpha\beta) \qquad \text{(Eq. 11)}$$

$$X_1 = 1.0 \qquad \text{(Eq. 12)}$$

$$X_2 = t_i - t_1 \qquad \text{(Eq. 13)}$$

$$X_3 = \int_{t_1}^{t_i} C\,dt \qquad \text{(Eq. 14)}$$

$$X_4 = \int_{t_1}^{t_i} X_3\,dt \qquad \text{(Eq. 15)}$$

In deriving Eqs. 8 and 9, the following identities apply:

$$\int_0^{t_i} C\,dt = \int_0^{t_1} C\,dt + \int_{t_1}^{t_i} C\,dt \qquad \text{(Eq. 16)}$$

$$\int_0^{t_i}\int_0^{t_i} C\,dt^2 = \int_0^{t_1}\int_0^{t_1} C\,dt^2 + (t_i - t_1)\cdot\int_0^{t_1} C\,dt + \int_{t_1}^{t_i}\int_{t_1}^{t_i} C\,dt^2 \qquad \text{(Eq. 17)}$$

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