mg/kg in rats, indicating that saturation occurred and caused a decrease in the distribution from plasma. There was no change in the bile-to-liver concentration ratio over the same dose range, suggesting that nonlinear plasma elimination was due to saturable liver uptake only.

The data from the liver studies support the data of Takada (4) because a partial saturation of the transfer process (uptake) from the plasma to the liver occurred at the higher dose (Fig. 4). It has been previously hypothesized that the saturability of the uptake process is due to the limited binding capacity on the hepatic intracellular macromolecules (Y and Z), which have been shown to be instrumental in the hepatobiliary transport of I (4, 5). Nagashima *et al.* (15) suggested that saturable tissue binding can be responsible for the type of nonlinearity observed with this data. If the macromolecular binding sites for I become saturated at higher doses, the fraction of free drug in the liver should increase. Classically, only free drug can transfer across membranes. Therefore, I could then transfer back into plasma resulting in an increase in the apparent elimination (β-phase) half-life. The same result would occur if a very tight binding site for I was saturated and more dye was bound to a protein for which it normally has less affinity. The data from this study and from previous studies (3–6) support the hypothesis that the plasma nonlinearity is due to the saturation of the hepatic protein binding sites for I.

The saturation of the liver at higher doses of I was reflected in the bile as well (Fig. 5). Takada *et al.* (4) reported that the excretion of I in bile was not saturable over the dose range of 11.2–66.7 mg/kg because the bile-to-liver concentration ratio remained unchanged. Therefore, the observed differences in the cumulative amount excreted and in the excretion rate of I in the bile after 5.6- and 11.2-mg/kg doses must be a direct result of the saturation of the uptake process in the liver (Fig. 5). The same result occurred with the infusion data; *i.e.*, the amount of I excreted per hour in the bile plateaued at 8.5 mg/hr at the two highest infusion rates, indicating a dependence on the amount of I present in the liver (Fig. 6). Overall, the excretion of I in bile was linear.

When liver injury was induced by carbon tetrachloride administration, a significant reduction in the rate of excretion of I in bile occurred (Table II). The excretion of I in bile in carbon tetrachloride-treated rats decreased to the extent that it is doubtful that a linear model would apply. The extent of parameter changes that would be calculated from the data may be a reflection of both the liver damage produced by the carbon tetrachloride treatment and the nonlinear behavior of I.

In summary, the disposition of I in the rat was characterized. The apparent first-order elimination kinetics of I in plasma were nonlinear because the liver uptake process was saturable. This was evident from the increase in elimination half-life and disproportionate increase in area under the plasma concentration–time curve with increase in dose. The nature of the saturation was not determined in this study, but it does appear that it is not a Michaelis–Menten effect because I is neither metabolized (2) nor does it demonstrate the appropriate plasma concentration–time profiles with ascending doses (*i.e.*, parallel slopes at concentrations below K_m). It is also not a facilitated transport effect because metabolic inhibitors do not influence the uptake of I by liver cells (4). The amount of I excreted in bile was dependent on the amount present in the liver reflecting the uptake saturability.

These findings could be applied to other nonmetabolizing organic anions and, with adaptations, to compounds that are metabolized. Further work regarding the nature of the saturation and regarding the disposition in higher animals is suggested in order to completely evaluate the use of I as a general index for hepatobiliary function.

REFERENCES

(1) P. C. Hirom, P. Millburn, R. L. Smith, and R. T. Williams, *Biochem. J.*, **129**, 1071 (1972).

(2) T. H. Kim and S. K. Hong, *Am. J. Physiol.*, **202**, 174 (1962).

(3) R. B. Smith, L. McWhorter, and J. W. Triplett, *Int. J. Nucl. Med. Biol.*, **7**, 37 (1980).

(4) K. Takada, Y. Mizobuchi, and S. Muranishi, *Chem. Pharm. Bull.*, **22**, 922 (1974).

(5) K. Takada, M. Ueda, M. Ohno, and S. Muranishi, *Chem. Pharm. Bull.*, **22**, 1477 (1974).

(6) K. Takada, S. Muranishi, and H. Sezaki, *J. Pharmacokinet. Biopharm.*, **2**, 495 (1974).

(7) S. K. Lin, A. A. Moss, R. Motson, and S. Riegelman, *J. Pharm. Sci.*, **67**, 930 (1978).

(8) P. Chelvan, J. M. T. Hamilton-Miller, and J. N. Brumfit, *Br. J. Clin. Pharmacol.*, **8**, 233 (1979).

(9) W. A. Colburn, P. C. Hirom, R. J. Parker, and P. Millburn, *Drug Metab. Dispos.*, **1**, 100 (1979).

(10) R. A. Zito and P. R. Reid, *J. Clin. Pharmacol.*, **21**, 100 (1981).

(11) K. Stoeckel, P. J. McNamara, A. J. McLean, P. duSouich, D. Lalka, and M. Gibaldi, *J. Pharmacokinet. Biopharm.*, **8**, 483 (1980).

(12) C. M. Metzler, G. L. Elfring, and A. J. McEwen, "A Users Manual for NONLIN and Associated Programs," The Upjohn Co., Kalamazoo, Mich., 1974.

(13) D. Perrier, J. I. Ashley, and G. Levy, *J. Pharmacokinet. Biopharm.*, **1**, 231 (1973).

(14) F. Barbier and G. A. DeWeerdt, *Clin Chim. Acta*, **10**, 549 (1964).

(15) R. Nagashima, G. Levy, and E. J. Sarcione, *J. Pharm. Sci.*, **57**, 1881 (1968).

ACKNOWLEDGMENTS

The authors thank Mrs. Judy Webster for typing this manuscript.

Picrotoxin-Like Lactones

JAMES D. McCHESNEY *x and ALAN F. WYCPALEK

Received April 26, 1982, from the *Department of Medicinal Chemistry, School of Pharmacy, The University of Kansas, Lawrence, KS 66045.* Accepted for publication July 23, 1982.

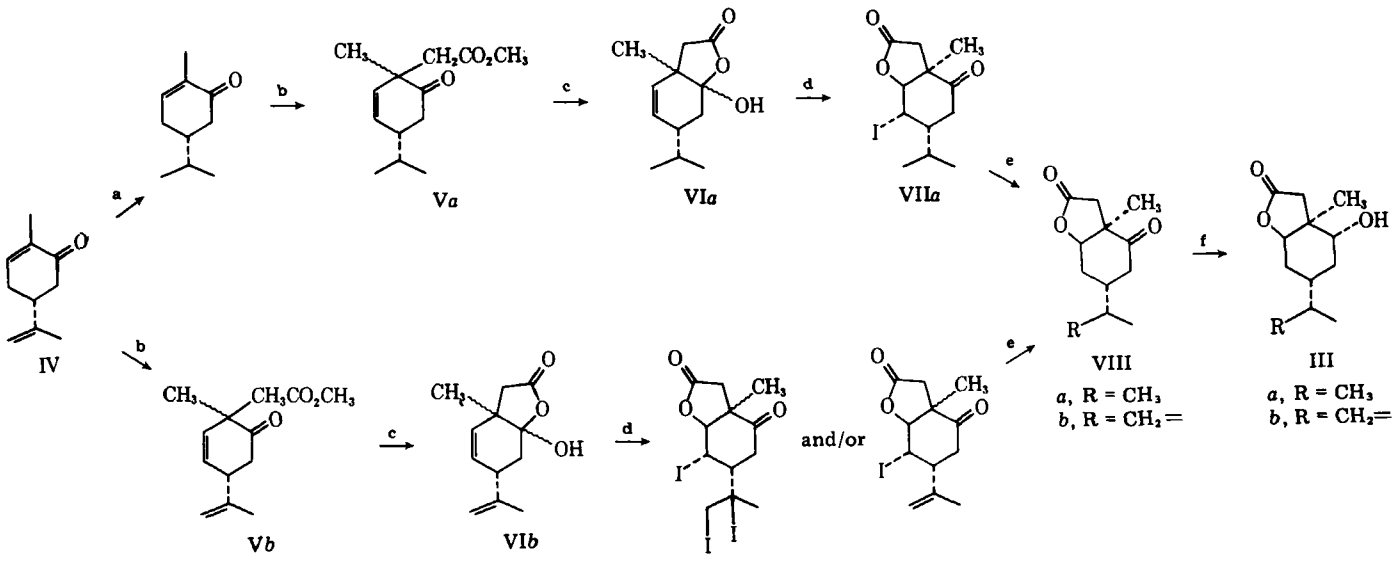
*Present address: Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677.

Abstract □ Preparation of some simple lactone analogues of picrotoxin and their biological evaluation is reported. Certain analogues possessed activity, but at potencies insufficient to warrant further work.

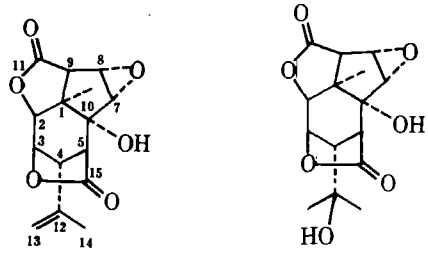
Keyphrases □ Picrotoxin—lactone analogue synthesis, CNS activity □ Analogues—of picrotoxin, synthesis, CNS activity □ CNS activity—of picrotoxin lactone analogues, synthesis

Although picrotoxin [an equimolar mixture of picrotin (I) and picrotoxinin (II)] was used as early as the 18th century as a fish toxin and clinically in humans during the 1930s and 1940s, only in the last decade has an understanding of its mode of action as a CNS stimulant begun to emerge. It was first recognized as a γ-aminobutyric acid antagonist (1), but more recently its mechanism of antagonism has been shown to be inhibition of chloride ion permeability in neuronal preparations (2). Jarboe and coworkers (3) have examined aspects of the structure–activity relationship (SAR) of these materials and concluded that in order to possess activity, certain features were required: (*a*) a free bridgehead hydroxyl group, (*b*) a lactone ring connecting carbons 3 and 5, and (*c*) an isopropenyl group (which appears to govern potency).

0022-3549/83/1000-1131$01.00/0

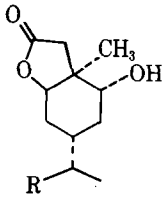


Scheme I—Synthesis of lactones III. Key: (a) *tris*-triphenylphosphine rhodium chloride–hydrogen; (b) potassium-*tert*-amylate–methyl bromoacetate; (c) potassium hydroxide; (d) iodine–potassium iodide, sodium bicarbonate; (e) triphenyltin hydride; (f) sodium borohydride.



I II

To ascertain if a simpler agent might possess picrotoxin-like activity, *i.e.*, if the lactone ring connecting carbons 3 and 5 is essential while the lactone connecting carbons 2 and 11 plays no role (as previously suggested), we set about to prepare analogous lactones (III) and determine if they showed CNS stimulatory activity similar to picrotoxin in rodents.

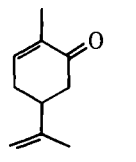


III

a R = CH_3
b R = CH_2=

RESULTS AND DISCUSSION

The most convenient preparation of the target compounds with asymmetric centers of the same configuration as picrotoxin seemed to reside in choosing a starting material with at least some of these centers already established. Then one may rely on the particular features of the molecule, such as steric factors and conformation, to control the course of reactions which would be, thereby, regio- and stereoselective. With these factors in mind, it can be seen that the most suitable starting material is the known natural product (−)-carvone (IV).



IV

The desired lactones (III) should be available from carvone *via* introduction of a suitable two-carbon side chain α to the ketone, lactonization, and subsequent reduction of the ketone function. A convenient approach for the introduction of the two-carbon side chain was expected to be the alkylation of the enolate generated by base abstraction of a γ proton of an α,β-unsaturated ketone. There is ample precedent for this expected alkylation in polycyclic systems (4). However, Theobald (5) reported dimerization of carvone under the projected reaction conditions. Initial attempts in our laboratory at direct base-catalyzed alkylation of carvone afforded only over-alkylated products. Finally, careful investigation of the effects of the nature of the alkoxide anion, metal cation, solvent, and temperature conditions[1] of the base-catalyzed alkylation afforded a convenient direct procedure. It was found that alkylation of carvone with potassium *tert*-amylate–methyl bromoacetate in a mixture of dimethoxyethane and dimethylformamide afforded acceptable yields of the desired product, methyl-2-[1(*R*,*S*)-methyl-2-oxo-4(*S*)-isopropenyl-5-cyclohexenyl]acetate (V*b*). Alternatively, prior reduction of the isopropenyl group of carvone followed by alkylation under the same conditions afforded V*a*. The keto esters were then hydrolyzed in base producing lactols VI, which were lactonized under iodolactonization conditions to a mixture of iodo lactones VII. Iodo lactone VII*b* was not characterized fully because it darkened rapidly on isolation, apparently due to the presence of triiodo lactone formed by iodination of the isopropenyl group during the lactonization sequence. The iodo lactones were deiodinated by reduction with triphenyltin hydride and the resultant keto lactones VIII were reduced to the hydroxy lactones III with sodium borohydride (Scheme I).

The structural assignments were based on the following:

1. The sequence was initiated with a starting material of known absolute configuration. Selective reduction of the isopropenyl group should not modify any centers of stereochemistry.
2. The base-catalyzed alkylation would be expected to produce a mixture with β-alkylation (*trans* to the isopropyl and isopropenyl group) predominating (6).
3. Hydrolysis of the acetate ester mixture and iodolactonization produced a mixture (3:1 ratio) of γ-lactones, showing carbonyl absorption at 1780 cm^{-1} in the IR spectrum.
4. The major iodo lactone isomer was assigned structure VII based on its carbonyl absorption and by an examination of the coupling pattern of the methine hydrogen α to the lactone ether linkage, showing one proton doublet, δ 5.1 ppm (J = 4 Hz). This proton is clearly diequatorially coupled to the adjacent methine α to the iodine function. This is consistent with the usual transdiaxial iodolactonization mechanism.

[1] H. N. Edwards; unpublished work.

5. Deiodination does not alter stereochemistry at any centers, so structure VIII represents the keto lactones.

6. Sodium borohydride reductions of cyclohexanones proceed with introduction of hydrogen from the less hindered face, in this case from the β face, to produce hydroxy lactones III.

Biological evaluation was made with a screening procedure which attempts to selectively utilize available techniques to identify major types of central nervous system (CNS) activity. It is not definitive, but rather a qualitative evaluation. Generally, it is a comparison of activity between an unknown and a control vehicle. The detection screen employs direct visual observation of behavior plus measures of simple reflex activities. In this particular case attention was given to muscle tonus. The animals were placed in an observation arena, which is a large metal tray lined with bedding[2]. The animals were provoked to walk and were gently handled to judge body tonus. Compounds III*a*, III*b*, VIII*a*, and VIII*b* were evaluated at doses of 1, 10, 100 (and higher, if quantities permitted) mg/kg in female Wistar rats. Compound III*a* produced no effect at doses of ≤200 mg/kg (the highest dose tested). Compound III*b* produced no effect at doses of ≤100 mg/kg, but caused death within 24 hr at 150 mg/kg, the highest dose evaluated. It is unknown if picrotoxin-like convulsions were produced. At doses of <100 mg/kg, ketolactone VIII*a* caused no observable effect, but caused the animal to die within 4 days after a 100-mg/kg dose. At a dose of 200 mg/kg this ketolactone produced onset of tonic convulsions within 3 min followed by clonic convulsions by 10 min, with pilorection and salivation. The animals died within a 20-min period. Keto lactone VIII*b* produced no effects at doses <100 mg/kg, but at a dose of 125 mg/kg (the highest dose tested) the animals died within 12–15 hr. It is unknown if convulsions were produced. Because doses >100 mg/kg were required to produce biological effects, the potency of this series was not sufficient to develop further.

EXPERIMENTAL[3]

Preparation of Potassium-*tert*-amyloxide—To 60 ml of *tert*-amyl alcohol (44.5 g, 0.55 mole) contained in 500 ml of dimethoxyethane under argon was added potassium (23.45 g, 0.6 g-atom), and the mixture was heated to reflux for 2 days. The contents were allowed to cool to room temperature, and the reflux condenser was replaced with a rubber septum. The resulting stock solution was standardized by titration with 2.42 × 10^{-2} *N* potassium acid phthalate solution (phenophthalein as indicator) before each base reaction was carried out.

Alkylation Reactions Using Potassium-*tert*-amyloxide—To 0.1 mole of enone in 250 ml of dimethylformamide stirring in an ice bath under argon was added 0.086–0.14 mole of freshly prepared base solution over a period of 10 min. To the resultant mixture was added 0.2 mole of alkylating agent, and the contents were allowed to stir an additional 45 min at room temperature. The reaction was quenched with ice water and extracted with ether. The ether layer was washed 5 times with equal volumes of water and dried over sodium sulfate. The ether was removed, and the resulting oil was chromatographed or distilled.

Methyl 2-[1(*R,S*)-Methyl-2-oxo-4(*S*)-isopropenyl-5-cyclohexenyl]acetate, (V*b*)—*l*-Carvone (30.04 g, 0.20 mole) in 500 ml of dimethylformamide was alkylated with 62 ml of potassium-*tert*-amylate base solution (0.20 eq) and methyl bromoacetate (61.2 g, 0.40 mole). The resulting oil was distilled to give (V*b*) (8.6 g, 20%): bp 86–88° (0.05 mm); IR (neat) 1745, 1720, and 890; NMR ($CDCl_3$) 1.22 (3, s, CH_3), 1.78 (3, s, vinyl CH_3), 3.62 (3, s, OCH_3), 4.81 (2, m, $=CH_2$), and 5.73 ppm (2, m, H—C=C—H); $M^{\oplus}$ *m/z* 222; n_D^{22} 1.4955.

***l*-Carvotanacetone, 5(*R*)-Isopropyl-2-methyl-2-cyclohexanone**—The procedure used was that of Birch and Walker (7). Modifications were made in the percent of catalyst and the hydrogenation apparatus used. To *l*-carvone (80 g, 0.53 mole) in 150 ml of absolute ethanol contained in a Parr bottle was added *tris*-triphenylphosphine rhodium chloride. The hydrogenation was carried out at room temperature and 30 psi over a period of 6 hr. The bottle was refilled with hydrogen twice during this period. The ethanol was removed, and the crude oil was distilled to produce *l*-carvotanacetone (77.4 g, 95%): bp 54–60° (0.4–0.6 mm); IR (neat) 1680, 1380, and 1365; NMR (CCl_4) 0.72 (6, d, *J* = 6 Hz, 2 CH_3), 1.72 (3, s, CH_3), and 6.6 ppm (1, br s, =CH); n_D^{28} 1.4760 [lit (8) bp 227–228°; n_D^{19} 1.4822].

Methyl 2-[1(*R,S*)-Methyl-2-oxo-4(*R*)-isopropyl-5-cyclohexenyl]acetate, (V*a*)—*l*-Carvotanacetone (15.2 g, 0.10 mole) in 250 ml of dimethylformamide was alkylated with 100 ml of potassium-*tert*-amylate solution (0.11 eq) and methyl bromoacetate (30.6 g, 0.20 mole). Chromatography (alumina; 10% ethyl acetate in cyclohexane) produced V*a* (6.75 g, 35%): bp 86–88° (0.075 mm); IR (neat) 1745, 1720, 1387, 1368, and 735; NMR ($CDCl_3$) 0.99 (6, d, *J* = 6 Hz, 2 CH_3), 1.2 (3, s, CH_3), 3.68 (3, s, OCH_3), and 5.84 ppm (2, m, HC=CH); $M^{\oplus}$, *m/z* 224; n_D^{24} 1.4744.

Ester Hydrolyses—The procedure used was that of Allen and Kalm (9) modified by shortening the reaction time to 2 hr. To 1 mmole of the keto ester in 2 ml of 95% ethanol was added 1.5 mmoles solid 85% KOH, and the mixture was heated to reflux for 2 hr. The ethanol was removed by evaporation, and the resulting oil was diluted with 5 volumes of ice water and acidified (pH 1) with 5 *N* sulfuric acid. The organic portion was extracted with ether, and the ether extract was dried over sodium sulfate.

2-[1(*R,S*)-Methyl-2-oxo-4(*S*)-isopropenyl-5-cyclohexenyl]-acetic acid-γ-lactol, (VI*b*)—The keto ester (V*b*) (6.3 g, 28 mmoles) in 55 ml of 95% ethanol was hydrolyzed with 85% KOH (3.0 g, 45 mmoles). The ether was removed to give 5.8 g of (VI*b*): IR (neat) 3400, 1780–1720 (br), 1650, and 890; NMR (CCl_4) 1.21 (3, s, CH_3), 2.75 (3, s, CH_3), 4.79 (2, m, $=CH_2$), and 6.6 ppm (2, m, HC=CH); $M^{\oplus}$, *m/z* 208; n_D^{26} 1.5088.

2-[1(*R,S*)-Methyl-2-oxo-4(*R*)-isopropyl-5-cyclohexenyl]-acetic acid-γ-lactol, (VI*a*)—The keto ester V*a* (4.92 g, 22 mmoles) in 40 ml of 95% ethanol was hydrolyzed with 85% KOH (2.2 g, 33 mmoles). The ether was removed affording 4.2 g of (VI*a*): IR (neat) 3440, 1775, and 1720; NMR (CCl_4) 0.95 (6, d, *J* = 6 Hz, 2 CH_3, 1.23 (3, s, CH_3), 5.6 (2, m, HC=CH), and 6.9 ppm (1, s, lactol H); $M^{\oplus}$, *m/z* 210; n_D^{24} 1.4990.

Procedure for Iodolactonization—The procedure used was that of Van Tamelen and Shamma (10). The only modification made was a one-fold increase of iodine and potassium iodide in the doubly unsaturated lactol. To a solution of 5 mmoles of the lactol in 30 ml of 0.5 *N* $NaHCO_3$ was added 30 mmoles of potassium iodide in 15 ml of water and 10 mmoles of iodine. The mixture was allowed to stand in the dark for 24 hr. The contents of the reaction was transferred to a separatory funnel and diluted with ether. The mixture was shaken with a saturated solution of sodium thiosulfate until two almost colorless phases were obtained. The ether layer was concentrated and the iodo lactones were allowed to crystallize.

2-[1 (*R*)-Methyl-2-oxo-4 (*S*)-(1′,2′-diiodo)-isopropyl-5 (*S*)-iodo-6(*S*)-hydroxycyclohexyl-acetic acid-γ-lactone or 2-[1 (*R*)-Methyl-2-oxo-4 (*R*)-isopropenyl-5 (*S*)-iodo-6 (*S*)-hydroxycyclohexyl]-acetic acid-γ-lactone (VII*b*)—The lactol (VI*b*) (4.25 g, 20 mmoles) in 60 ml of 0.5 *N* $NaHCO_3$ was lactonized with iodine (20.0 g, 79 mmoles) and potassium iodide (2.0 g, 12 mmoles). The ether extract was concentrated, and the mixture was treated with triphenyltin hydride without purification.

2-[1 (*R*)-Methyl-2-oxo-4 (*R*)-isopropyl-5 (*S*)-iodo-6 (*S*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (VII*a*)—The lactol VI*a* (4.8 g, 23 mmoles) in 130 ml of 0.5 *N* $NaHCO_3$ was lactonized with iodine (10.44 g, 41 mmoles) and potassium iodide (2.16 g, 13 mmoles). Fractional crystallization afforded the major isomer, VII*a* (2.16 g, 36.5%): mp 113–114°; IR ($CHCl_3$) 1780 and 1712; NMR ($CDCl_3$) 0.98 (3, d, *J* = 6 Hz, CH_3), 1.05 (3, d *J* = 6 Hz, CH_3), 1.55 (3, s CH_3), 4.79 (1, m, I—C—H), and 5.15 ppm (1, d *J* = 2 Hz, O—C—H); $M^{\oplus}$, *m/z* 336; $[\alpha]_D^{25}$($CHCl_3$) −13.5°.

Anal.—Calc. for $C_{12}H_{17}IO_3$: C, 42.86; H, 5.06. Found: C, 43.04; H, 5.21.

Preparation of Triphenyltin Hydride—The preparation used was that of Kuivila (11) without modification. To 150 ml of dry ether containing lithium aluminum hydride (1.56 g, 41 mmoles) was added slowly triphenyltin chloride (32.5 g, 100 mmoles). The mixture was stirred at room temperature under argon for 3 hr and then hydrolyzed with 100 ml of ice water while cooling in an ice bath. The ether layer was washed twice with ice water then dried over magnesium sulfate. The ether was removed, and the hydride was distilled rapidly with a bath preheated to 200°; yield 19.5 g (69%); bp 144–152° (0.2–0.3 mm) [lit. (11) 162–168° (0.5 mm)].

Triphenyltin Hydride Reduction of Iodolactones—The procedure used was that of Kuivila (11). The only modification which was made was a onefold increase of the hydride in the iodo lactones of the doubly unsaturated γ-lactol. To 1 mmole of the iodo lactone contained in 10 ml of dry benzene stirring under argon was added 1 mmole of triphenyltin hydride. The mixture was stirred at room temperature overnight, and then the benzene was removed. The resulting product was either fractionally sublimed or fractionally distilled.

[2] Sanicel.

[3] All melting points were taken on a Thomas-Hoover Unimelt and are corrected. Analyses were performed by either Midwest Microlab, Inc., Indianapolis, Ind., or on an F and M Model 185, C, H, N, Analyzer, University of Kansas. IR data were recorded on Beckman IR-8, IR-10, and IR-33 spectrophotometers and are reported in cm^{-1}. ^1H-NMR data were recorded on Varian A-60, A-60A, and T-60 analytical spectrometers with tetramethylsilane as the internal standard. ^1H-NMR data are reported as δ-values (ppm). Mass spectra were recorded on a Varian-Atlas CH-5 at 70 eV at a resolution of ~4000.

2-[1(*R*)-Methyl-2-oxo-4(*R*)-isopropenyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (VIII*b*)—The iodo lactone(s) VII*b* (3.8 g) in 100 ml of dry benzene was reduced with triphenyltin hydride (6.72 g, 0.02 mole). Distillation (150–154° and 0.1 mm) provided VIII*b* (975 mg, 72% based on monoiodo starting material, 91% based on triiodo starting material): IR ($CHCl_3$) 1795 and 1720; NMR ($CDCl_3$) 1.35 (3, s, CH_3), 1.75 (3, s, CH_3), 4.68 (1, t, J = 2 Hz, O—C—H), and 4.82 ppm (2, m, $=CH_2$); $M^{\oplus}$, m/z 208; $[\alpha]_D^{26}$ ($CHCl_3$) +114.2°.

Anal.—Calc. for $C_{12}H_{16}O_3$: C, 69.20; H, 7.68. Found: C, 68.95; H, 7.79.

2-[1(*R*)-Methyl -2- oxo-4(*R*)-isopropyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (VIII*a*)—The iodo lactone VII*a* (1.0 g, 3 mmoles) in 30 ml of dry benzene was reduced with triphenyltin hydride (0.98 g, 2.8 mmoles). The benzene was removed, and the resulting gum was fractionally sublimed [67° (0.05 mm)] to yield VIII*a* (200 mg, 32%): IR ($CHCl_3$) 1785 and 1715; NMR ($CDCl_3$) 0.95 (6, d, J = 6 Hz, 2 CH_3), 1.35 (3, s, CH_3), and 4.67 ppm (1, t, J = 2 Hz, O—C—H); $M^{\oplus}$, m/z 210; $[\alpha]_D^{26}$ ($CHCl_3$) +127°; mp 84–85°.

Anal.—Calc. for $C_{12}H_{18}O_3$: C, 68.55; H, 8.57. Found: C, 68.33; H, 8.52.

Sodium Borohydride Reductions—The procedure used was that of Elisberg *et al.* (12) modified by length of reaction time and solvent. To 0.5 mmole of the keto lactone in 30 ml of 95% ethanol stirring in an ice bath was added 1 mmole of sodium borohydride. Ice water was added to the mixture, the solution was extracted with ether three times, and the organic layer was dried over sodium sulfate.

2-[1(*S*)-Methyl-2(*R*)-hydroxy -4(*S*)- isopropenyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (III*b*)—The keto lactone (VIII*b*) (560 mg, 2.69 mmoles) in 40 ml of 95% ethanol was reduced with sodium borohydride (200 mg, 5.38 mmoles). Workup after a 6-hr reaction time and distillation produced III*b* (270 mg, 48%): IR (neat) 3500 and 1770; NMR ($CDCl_3$) 1.1 (3, s, CH_3), 1.74 (3, s, CH_3), 4.35 (1, t, J = 3 Hz, O—C—H), and 4.73 ppm (2, s, $=CH_2$); $M^{\oplus}$, m/z 210.

Anal.—Calc. for $C_{12}H_{18}O_3$: C, 68.59; H, 8.57. Found: C, 68.34; H, 8.63.

2-[1(*S*)-Methyl-2(*R*)-hydroxy-4(*S*)-isopropyl-6(*R*)-hydroxycyclohexyl]-acetic acid-γ-lactone, (III*a*)—The keto lactone, VIII*a* (315 mg, 1.5 mmoles) in 30 ml of 95% ethanol was reduced with sodium borohydride (113 mg, 3.0 mmoles). Workup after a period of 7 hr and distillation gave III*a* (130 mg, 41%): IR (neat) 3500 and 1775; NMR ($CDCl_3$) 0.85 (3, d, J = 5 Hz, CH_3), 1.1 (3, d, J = 4 Hz, CH_3), 1.22 (3, s, CH_3), and 4.3 ppm (1, s, O—C—H); $M^{\oplus}$, m/z 212.

Anal.—Calc. for $C_{12}H_{20}O_3$: C, 67.94; H, 9.43. Found: C, 67.85; H, 9.49.

REFERENCES

(1) G. A. R. Johnston, *Annu. Rev. Pharmacol. Toxicol.*, **18**, 269 (1978).

(2) M. K. Ticker and R. W. Olsen, *Biochim. Biophys. Acta*, **464**, 519 (1977).

(3) C. H. Jarboe, L. A. Porter, and R. T. Buckler, *J. Med. Chem.*, **11**, 729, (1968).

(4) H. D. House, "Modern Synthetic Reactions," 2nd ed., W. A. Benjamin, Menlo Park, Calif., 1972, chap. 9.

(5) D. W. Theobald, *Tetrahedron*, **23**, 2767 (1967).

(6) R. S. Matthews, S. J. Girgenti, and E. A. Folkers, *J. Chem. Soc., Chem. Comm.*, **1970**, 738.

(7) A. J. Birch and K. Walker, *J. Chem. Soc., C*, **1964**, 1894.

(8) O. Wallach., *Ann. Chem.*, **336**, **1901**, 1.

(9) C. F. Allen and M. J. Kalm, *Org. Syn. Coll.*, **4**, 608 (1963).

(10) E. E. Van Tamelen and M. Shamma, *J. Am. Chem. Soc.*, **76**, 2315 (1954).

(11) H. G. Kuivila, *Synthesis*, **1970**, 499.

(12) E. Elisberg, H. Vanderhaege, and T. F. Gallager, *J. Am. Chem. Soc.*, **74**, 2814 (1952).

ACKNOWLEDGMENTS

Taken in part from the dissertation presented by A. F. Wycpalek to the Graduate School of the University of Kansas in partial fulfillment of the requirements for the Doctor of Philosophy degree in 1972.

The authors gratefully acknowledge the financial support of the National Institutes of Health. We wish to thank the Department of Pharmacology and Toxicology, University of Kansas Medical Center, Kansas City, Kansas through whose cooperation the biological evaluations were made.

Influence of Ionic Strength on Rectal Absorption of Gentamicin Sulfate in the Presence and Absence of Sodium Salicylate

JOSEPH A. FIX ˣ, PAULA S. LEPPERT, PATRICIA A. PORTER, and LARRY J. CALDWELL

Received November 20, 1981, from *INTERx Research Corporation*, a subsidiary of *Merck Sharp & Dohme Research Laboratories, Lawrence, KS 66044.* Accepted for publication August 16, 1982.

Abstract □ The rectal absorption of gentamicin sulfate in rats, both in the presence and absence of sodium salicylate, was facilitated by the use of high ionic strength aqueous formulations. The relative order of effectiveness in promoting gentamicin absorption was sodium dihydrogen phosphate ≃ sodium chloride ≫ potassium chloride, indicating a preferential effect of sodium ions. The increased gentamicin bioavailability in response to sodium salicylate adjuvant activity appeared to be independent of and additive to the increased gentamicin absorption due to high ionic strength conditions. The inability of sorbitol to increase gentamicin bioavailability above control levels indicated that elevated osmotic pressure was not a major determinant of rectal gentamicin absorption.

Keyphrases □ Gentamicin—rectal absorption, effect of ionic strength and specificity, sodium salicylate adjuvant □ Sodium salicylate—as adjuvant, rectal absorption of gentamicin, effect of ionic strength and specificity □ Absorption, rectal—gentamicin, sodium salicylate adjuvant, effect of ionic strength and specificity

Sodium salicylate has been reported to enhance the rectal absorption of water-soluble compounds (1, 2). Two types of formulations were used in the salicylate studies, an aqueous microenema and a fatty-base suppository. The influence of variations in ionic strength and ionic specificity on salicylate-enhanced rectal absorption has not been thoroughly examined in either formulation.

The effect of sodium concentration on absorption of fluid, as classically illustrated by the active transport of sodium from the large intestine and the concomitant water

0022-3549/83/1000-1134$01.00/0