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STEREOCHEMISTRY OF THE JABORANDI ALKALOIDS

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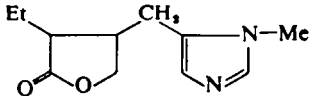
Abstract—The relative configurations of pilocarpine and isopilocarpine, the major alkaloids of jaborandi leaves, have been established as *cis* and *trans*, respectively. Degradation of their oxidation product, homoisopilopic acid, to an optically active 2,3-diethylbutane-1,4-diol proves the *trans* sterochemistry of the *iso* series. Assignment of absolute configuration is based on (a) conversion of homoisopilopic acid to (2R:3R) 2,3-diethyl-γ-butyrolactone and (b) interrelation of isopilopic acid with R(+)-ethylsuccinic acid.

THE major alkaloids of jaborandi leaves (from shrubs of South American *Pilocarpus* species), of interest because of their valuable pharmacological properties, are pilocarpine and isopilocarpine, stereoisomers of structure I, and pilocarpidine, the



I

N-desmethyl derivative of pilocarpine.[1] Pilocarpine is a widely-studied[2] peripheral stimulant of the parasympathetic system, employed topically as a miotic and internally as a diaphoretic in the treatment of nephritis. Isopilocarpine is qualitatively similar in its physiological effects, but considerably less active.

The structures of pilocarpine and its isomer were solved by Jowett[3] in 1900, and both were synthesized[4] by several routes during the 1930's. Both the extensive degradative and synthetic studies failed to provide a definitive answer to the stereochemistry of these bases. Since the two isomers differ in physiological activity, the relative and absolute configurations of the alkaloids have been determined.

Relative configuration. From a consideration of the optical rotations of derivatives of the normal and *iso* series. Preobrashenski *et al.*[5] suggested that in pilocarpine the

[1] For an excellent review of the jaborandi alkaloids, see A. R. Battersby and H. T. Openshaw, *The Alkaloids* (Edited by R. H. F. Manske and H. L. Holmes) Vol. III; p. 201. Academic Press, New York, N.Y. (1953).

[2] J. M. van Rossum, M. J. W. J. Cornelissen, C. Th. P. de Groot and J. A. Th. M. Hurkmans, *Experentia* **16**, 373 (1960).

[3] H. A. D. Jowett, *J. Chem. Soc.* **77**, 473, 851 (1900).

[4] *a* W. A. Preobrashenski, N. A. Preobrashenski and A. F. Wompe, *Ber. Dtsch Chem. Ges.* **66**, 1187 (1933); *b* N. A. Preobrashenski, W. A. Preobrashenski, A. F. Wompe and M. N. Schtschukina, *Ibid.* **66**, 1536 (1933); *c* A. N. Dey, *J. Chem. Soc.* 1057 (1937).

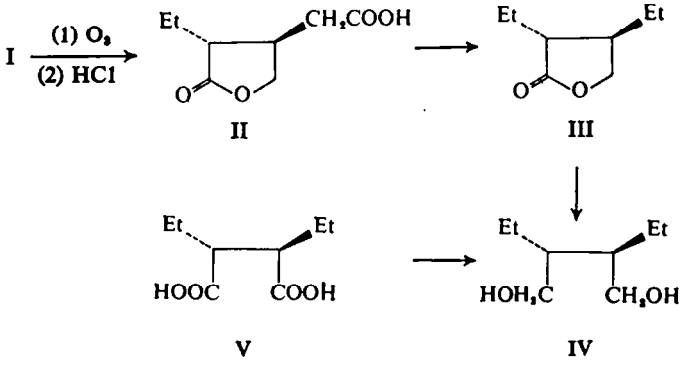
[5] N. A. Preobrashenski, A. M. Polyakova and V. A. Preobrashenski, *Bull. Acad. Sci. U.R.S.S.* 983 (1936).

two substituents on the lactone ring are *cis*. It is known that the two isomers are readily interconvertible, by heating the bases or their hydrochlorides, or by warming with alkali, and that isopilocarpine predominates at equilibrium. Additional studies comparing rates of ring closure of halogenated derivatives of the two series also support[6] the interference that the more stable *iso* series has the *trans* configuration.

In order to confirm this assignment, advantage was taken of the near-symmetry of the carbon skeleton of pilocarpine to prepare a completely symmetrical derivative in which the presence or absence of optical activity would provide an unambiguous answer to the question of configuration. The starting material, homoisopilopic acid[7] (II) a known oxidation product of the *iso* series, was prepared by ozonolysis of pilocarpine followed by vigorous acid hydrolysis of the amide produced; epimerization to the *iso* acid occurs during hydrolysis.

The two substituents on the lactone ring of II were made identical by converting the carboxyl group to methyl. For this purpose, a mixture of II and a tenfold excess of acetic acid was subjected to Kolbe electrolysis. Fractionation of the neutral products gave (+) *trans*-2,3-diethyl-γ-butyrolactone (III), whose structure and stereochemistry were shown by IR comparison with an authentic sample.[8] Finally, III was reduced with LAH to (+) 2,3-diethylbutane-1,4-diol (IV), identical with the (+) diol prepared by hydride reduction of (+) α,β-diethylsuccinic acid (V). This conversion of II to an optically active form of IV is an unequivocal proof of the *trans* relation of the substituents in II. Consequently, isopilocarpine and the other members of the *iso* series are *trans*-2,3-disubstituted butyrolactones, while pilocarpine has the *cis* configuration.

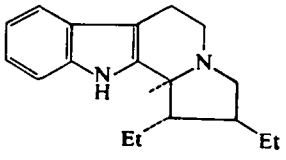
Absolute configuration. The recent work of Nagarajan *et al.*[8] revealed the absolute configuration of the degradation products III and IV. From (−) III these authors



prepared (−) 16α-strychindol (VI), a degradation product of strychnine. Since the absolute configuration of strychnine has been established by anomalous X-ray dispersion,[9] III may be related to it as a primary standard. In addition, the absolute

[6] S. I. Zav'yalov, *Dokl. Akad. Nauk, S.S.S.R.* **82**, 257 (1952).
[7] H. A. D. Jowett, *J. Chem. Soc.* **79**, 1331 (1901).
[8] K. Nagarajan, Ch. Weissmann, H. Schmid and P. Karrer, *Helv. Chim. Acta* **46**, 1212 (1963). We are indebted to Prof. Schmid for sending us the IR spectrum of lactone III.
[9] A. F. Peerdeman, *Acta Cryst.* **9**, 824 (1956).

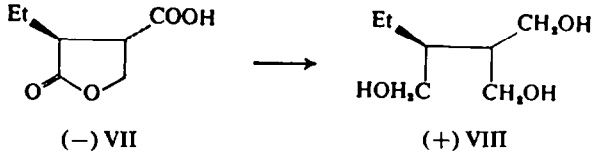
configuration of α,β-diethylsuccinic acid (V) determined by the quasi-racemate method,[10] shows that the dextrorotatory compounds III, IV, and V all have the (2R:3R) configuration.



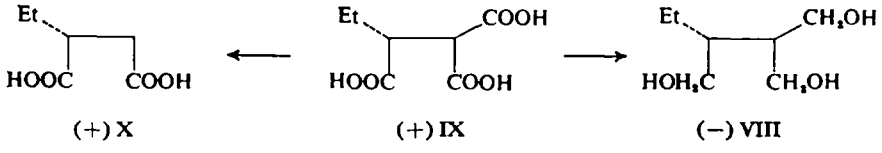
VI

Before these correlations were available, independent proof of absolute configuration was obtained from isopilopic acid (VII), another degradation product of the *iso* series. LAH reduction of (−) VII (the enantiomer of the acid obtained by oxidation of I) gave the triol VIII, characterized as its crystalline (+) tri-*p*-nitrobenzoate. The enantiomeric triol resulted from hydride reduction of (+) butane-1,1,2-tricarboxylic acid (IX). This acid, prepared by resolution of the racemic acid with quinine, was decarboxylated by heating to R(+)-ethylsuccinic acid.[11] This sequence of reactions establishes the (2S) configuration for (−) VII, and consequently the (2R) configuration for the acid obtained by oxidation of the alkaloids.

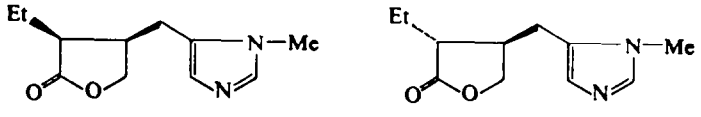
This investigation has therefore established the (2R:3R) configuration for isopilocarpine (Ib). Pilocarpine, epimeric at the asymmetric center adjacent to the carbonyl group, has the (2S:3R) configuration (Ia).



(−) VII (+) VIII



(+) X (+) IX (−) VIII



Ia Ib

EXPERIMENTAL

Pilocarpine hydrochloride. m.p. 200–201° (lit,[12] m.p. 200°) was obtained from the Inland Alkaloid Company.

Isopilocarpine was prepared as the nitrate, m.p. 156–157° (lit.[12] m.p. 158°) by isomerization of pilocarpine with NaOEt and precipitation with HNO_3.

Homoisopilopic acid (II). A chf solution of pilocarpine was ozonized according to the procedure

[10] A. Fredga and L. Terenius, *Acta Chem. Scand.* **18**, 2081 (1964).

[11] A. Fredga, *The Svedberg* (Memorial Volume) p. 261 (1944); *Chem. Abstr.* **39**, 1392 (1945).

[12] M. Petit and M. Polonovski, *Bull. Soc. Chim. Fr.* [3], **17**, 554, 702 (1897).

of Langenbeck.[13] The yield of recrystallized N-methylhomopilopamide, m.p. 110–111° (lit. m.p. 104°), was 79%.

A mixture of 55 g. of the amide and 550 ml of 2N HCl was heated for 3 hr at 100°, then cooled and extracted with chf. The extracts were dried and distilled, yielding 34·6 g (67%) of II, b.p. 221–227° (16 mm), n_D^{25} 1·47092, $[\alpha]_D^{25}$ +45·6° (c = 1·0 in H_2O). Jowett[7] reports b.p. 235–237° (20 mm) $[\alpha]_D$ +45·4°.

(2R:3R)-2,3-*diethyl-γ-butyrolactone* (III). A solution of 24·06 g of II, 84 g AcOH and 1·15 g AcONa in 150 ml MeOH was electrolysed at 1·0 amp and 55 v D.C., using 4 × 6 cm Pt electrodes separated by about 3–4 mm. The temp was maintained at 30–35° by cooling in an ice bath. After 43 hr the solution became yellow, the current dropped to 0·5 amp, and the pH rose to 8. Electrolysis was continued for 2 more hr, then the solution was concentrated at reduced press. The residue was taken up in ether, washed with $NaHCO_3$aq, dried and concentrated. Fractionation of the residue through an 8-in column gave 8·4 g of colorless lactone, b.p. 100–110° (10 mm), along with 4·3 g of material boiling at 112–162° (10 mm). The first fraction was redistilled, giving material of b.p. 104–112° (11 mm), n_D^{25} 1·4469, $[\alpha]_D^{25}$ +27·7° (c = 8·1 in chf). The IR spectrum was identical with that of an authentic sample,[8] for which the following constants have been reported: b.p. 104–105° (9 mm), n_D^{20} 1·4427, $[\alpha]_D^{24}$ +40·0° (c = 10·34 in chf).

meso-2,3-*Diethylbutane*-1,4-*diol*. A solution of 13·0 g *meso*-α,β-diethylsuccinic acid,[14] m.p. 198°, in 85 ml MeOH was esterified with diazomethane. The solvents were removed at reduced press, the residue taken up in dry ether and added dropwise to a stirred suspension of 5·0 g LAH in 200 ml ether. The mixture was refluxed overnight, cooled, treated with sat. Na_2SO_4aq and then with sufficient dil HCl to dissolve the salts. The layers were separated and the aqueous layer extracted several times with ether. The combined ether solutions were washed with brine, dried over $MgSO_4$, and concentrated. Distillation gave 8·0 g (73%) of the diol, b.p. (12 mm) 148·5–150°. (Found: C, 65·3; H, 12·3. $C_8H_{18}O_2$ requires C, 65·7; H, 12·4%.)

The *bis-phenylurethan* recrystallized from benzene–hexane, m.p. 118–118·5°. (Found: C, 68·58; H, 7·35; N, 7·25. $C_{22}H_{28}N_2O_4$ requires: C, 68·73; H, 7·34; N, 7·29%.)

The *bis*-3,5-*dinitrobenzoate* was recrystallized twice from EtOH, m.p. 128–130°. (Found: C, 49·61; H, 4·30; N, 10·32. $C_{22}H_{22}N_4O_{12}$ requires: C, 49·44; H, 4·15; N, 10·48%.)

(2R:3R)-2,3-*Diethylbutane*-1,4-*diol* (IV)

(a) *From* (+) α,β-*diethylsuccinic acid*. A solution of 6·7 g α,β-diethylsuccinic acid, $[\alpha]_D^{25}$ +37·2° (c = 2·90 in H_2O) (lit.[14] $[\alpha]_D$ +42°), in ether was esterified with diazomethane and reduced as described for the *meso* isomer. The diol, 2·52 g (45%), was collected at 148–150° (13 mm), $[\alpha]_D^{20}$ +13·6° (c = 6·61 in chf). Nagarajan *et al.*[8] report b.p. 105–110° (0·02 mm), $[\alpha]_D^{25}$ +12·9° (c = 5·76 in chf).

The *bis-phenylurethan*, recrystallized twice from ether–hexane, formed colorless needles, m.p. 105–106°, $[\alpha]_D^{25}$ −13·7° (c = 3·95 in EtOH) (Found: C, 68·78; H, 7·39; N, 7·35. $C_{22}H_{28}N_2O_4$ requires: C, 68·73; H, 7·34; N, 7·29%.)

The *bis*-3,5-*dinitrobenzoate*, after recrystallization from chf–EtOH, melted at 112–113°, $[\alpha]_D^{25}$ −13·2° (c = 4·28 in chf.) (Found: C, 49·21; H, 4·20; N, 10·21. $C_{22}H_{22}N_4O_{12}$ requires: C, 49·44; H, 4·15; N, 10·48%.)

(b) *From lactone* III. An ethereal solution of 2·11 g (+) III was reduced with 1·52 g LAH as described above. Distillation of the product gave 1·28 g diol, b.p. 109° (3 mm), n_D^{25} 1·4585 (lit.[8] n_D^{20} 1·4574).

The *bis-phenylurethan* was purified by chromatography on alumina, eluting with 1:2 benzene–hexane. Recrystallized twice from ether–hexane, it gave needles, m.p. 105·5–106°, not depressed by admixture with the derivative from part (a); $[\alpha]_D^{25}$ −13·9° (c = 6·51 in EtOH).

Resolution of butane-1,1,2-*tricarboxylic acid* (IX)

A solution of 42 g of racemic IX[15] in 150 ml of warm EtOH was treated with a solution of 71·5 g quinine in 170 ml warm EtOH; crystallization of the salt began within 10 min. The mixture was

[13] W. Langenbeck, *Ber. Dtsch Chem. Ges.* **57**, 2072 (1924).
[14] E. Berner and R. Leonardsen, *Liebigs Ann.* **538**, 1 (1939).
[15] G. Polko, *Liebigs Ann.* **242**, 115 (1887); C. A. Bischoff, *Ber. Dtsch Chem. Ges.* **24**, 2012 (1891).

kept for 9 hr, cooled in the refrigerator 2 hr, filtered and washed with a little EtOH. The solid, 96·5 g, was recrystallized from 500 ml EtOH and 250 ml H_2O to yield 41·7 g; this was recrystallized from a mixture of 700 ml EtOH, 100 ml acetone and 100 ml H_2O to yield 21·6 g. A final recrystallization from a mixture of 625 ml EtOH and 60 ml H_2O afforded 11·7 g of the *quinine salt*, m.p. 172–174° (dec) (Found: C, 63·24; H, 6·69; N, 5·58. $C_{27}H_{34}N_2O_8$ requires: C, 63·02; H, 6·66; N, 5·44%.)

The salt (11·3 g) was shaken with 200 ml 5% KOHaq and the liberated quinine extracted with chf. The aqueous solution was acidified with HCl, saturated with NH_4Cl, and extracted with five 100-ml portions of ether. The extracts were washed with sat. NH_4Claq, dried over $MgSO_4$, and evaporated, leaving 4·2 g of the triacid. Recrystallized from acetone, it melted at 148–149° (dec); $[\alpha]_D^{20}$ +71·5° (c = 14·87 in H_2O) the racemic acid has m.p. 141° (dec).[15]

Decarboxylation of triacid IX. Compound (+) IX (200 mg) was heated to 160–170° and kept at that temp for 10 min until decarboxylation was complete. The residue solidified on cooling. It was warmed with a few drops of water, then dried by distillation with benzene. Two recrystallizations from benzene–hexane gave colorless crystals of X, m.p. 96–96·5°, $[\alpha]_D^{20}$ +17·4° (c = 4·48 in H_2O). Berner and Leonardsen[14] report m.p. 96°, $[\alpha]_D^{5}$ +18·1° (H_2O).

LAH *Reduction of* (−) *isopilopic acid* (VII)

Racemic isopilopic acid (VII) was prepared by the procedure of Mehrotra and Dey[16] and resolved with strychnine.[17] The levorotatory acid had m.p. 104–105°, $[\alpha]_D^{21}$ −56·5° (c = 7·99 in H_2O); the reported[17] values are m.p. 105–105·5°, $[\alpha]_D^{25}$ −58·06° (H_2O).

A solution of 0·30 g of (−) isopilopic acid in 150 ml ether was stirred with 1·28 g LAH at room temp for 20 hr, and the excess reagent destroyed by the cautious addition of H_2O. $MgSO_4$ was added to coagulate the salts, the mixture was filtered, and the filter cake washed 5 times with ether. The combined filtrates were dried over $MgSO_4$, evaporated to dryness, and the residue converted to the tri-*p*-nitrobenzoate by boiling for a few min with 1·8 g *p*-nitrobenzoyl chloride in 15 ml pyridine. After standing overnight, the solution was taken up in chf and washed successively with HCl, H_2O, $KHCO_3aq$ and H_2O, then dried (Na_2SO_4) and concentrated. The residue crystallized from $CHCl_3$–EtOH, yielding 0·65 g of *the tri-*p*-nitrobenzoate of* VIII. It was recrystallized again from $CHCl_3$–EtOH, then 3 times from benzene, to give almost colorless crystals, m.p. 139–140° (with softening at 90–95° and resolidification at 110°), $[\alpha]_D^{23}$ +7·96° (c = 2·63 in chf) (Found: C, 56·74; H, 4·21; N, 7·05. $C_{28}H_{25}N_3O_{12}$ requires: C, 56·47; H, 4·23; N, 7·06%.)

LAH *reduction of* (+) IX. Compound IX was esterified with diazomethane and reduced with LAH and the triol (VIII) converted to the *tri-*p*-nitrobenzoate* as described above. After recrystallization from $CHCl_3$–EtOH, it crystallized from benzene, m.p. 138·5–139° (with previous softening and resolidification as noted for the enantiomer), $[\alpha]_D^{23}$ −7·23° (c = 3·00 in chf). The IR spectrum was superimposable with that of the (+) ester above.

Acknowledgement—This investigation was supported by a research grant (RG-6568) from the U.S. Public Health Service, to whom the authors express their thanks.

[16] J. K. Mehrotra and A. N. Dey, *J. Indian Chem. Soc.* **38**, 888 (1961).

[17] A. E. Tschitschibabin and N. A. Preobrashenski, *Ber. Dtsch Chem. Ges.* **63**, 460 (1930).