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ALKALOID STUDIES*—XX

ISOLATION AND STRUCTURE OF TWO NEW CACTUS ALKALOIDS PILOCEREDINE AND LOPHOCERINE

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Abstract—A detailed examination of the alkaloids of the cactus *Lophocereus Schottii* has resulted in the isolation of two new alkaloids, pilocerédine and lophocerine in addition to the previously described pilocereine; quaternary alkaloids were not encountered. Pilocerédine ($C_{30}H_{44}N_2O_4$) was shown to be diastereoisomeric with pilocereine and potassium-liquid ammonia cleavage of its methyl and ethyl ethers yielded the same cleavage products as the corresponding derivatives of pilocereine. Lophocerine was obtained in impure form from the phenolic alkaloid fraction and is assigned the structure 1-*iso*butyl-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydro*iso*quinoline since upon methylation it afforded the known 1-*iso*butyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro*iso*quinoline. Lophocerine appears to be the biogenetic precursor of pilocereine and pilocerédine.

AS POINTED out in an earlier paper[1] dealing with the structure elucidation of pilocereine (Ia) this cactus alkaloid is of unusual interest since its empirical formula $C_{30}H_{44}N_2O_4$ differs markedly from those (less than 13 carbon atoms) of the other alkaloids found among the *Cactaceae*.[2] Consequently an effort was made to isolate additional alkaloids from the cactus *Lophocereus Schottii* which appeared to be the richest source[3] of pilocereine.

The pilocereine-depleted "non-phenolic" alkaloid fraction was subjected to chromatography and after the separation of an additional quantity of pilocereine a second alkaloid was isolated (m.p. 165–166°) the analysis of which ($C_{30}H_{44}N_2O_4$ with two methoxyl and two N-methyl groups) coincided with that of pilocereine (Ia). The infra-red spectrum of this alkaloid was identical with that of pilocereine (m.p. 175–176°) when measured in chloroform solution but appreciable differences were noticed when the spectra were determined in nujol mull. Furthermore, the X-ray diffraction patterns§ were different. The possibility of polymorphism was excluded in the following manner: (a) the new alkaloid—pilocerédine—could be separated chromatographically from pilocereine; (b) the two alkaloids exhibited a marked

* Part XIX by M. Gorman, J. P. Kutney, P. J. Scheuer, N. Neuss and C. Djerassi *Tetrahedron* **1** (1957) is in the press.

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§ Kindly measured by Miss Ann Van Camp in the Eli Lilly Research Laboratories (Indianapolis, Indiana) through the courtesy of Dr. R. G. Jones.

[1] C. Djerassi, S. K. Figdor, J. M. Bobbitt and F. X. Markley *J. Amer. Chem. Soc.* **79**, 2203 (1957).

[2] For review see L. Reti in R. H. F. Manske and H. L. Holmes *The Alkaloids* Vol. IV, pp. 7–28, Academic Press, New York (1954).

[3] (a) G. Heyl *Arch. Pharm.* **239**, 451 (1901); (b) C. Djerassi, N. Frick and L. E. Geller *J. Amer. Chem. Soc.* **75**, 3632 (1953); (c) C. Djerassi, C. R. Smith, S. P. Marfey, R. N. McDonald, A. J. Lemin, S. K. Figdor and H. Estrada *Ibid.* **76**, 3215 (1954).

depression in melting point when mixed; (c) the two substances were not interconvertible by cross seeding in the same solvent; (d) as shown in Table 1, out of four derivatives, three exhibited very similar melting points but in each instance a marked mixture melting point depression was observed; (e) the respective acetates had very different melting points but even here the infra-red spectra were essentially identical when measured in carbon disulfide solution.

TABLE 1. COMPARISON OF MELTING POINTS OF PILOCEREINE,* PILOCEREDINE AND DERIVATIVES

Derivative	Pilocereine*	Piloceredine
Free base	175–176°	165–166°
Diperchlorate	216–217°	221–222°
Acetate	186–186.5°	133–134°
Methyl ether	133–134° and 153–155°	141–142°
Ethyl ether	147–148°	150–152°

* These constants were determined simultaneously with those of piloceredine and in some cases differ very slightly from those reported earlier.[1,8b]

Piloceredine, like pilocereine, was optically inactive* and the similarities in physical properties suggest that the former might be the possible diastereoisomer of pilocereine (Ia). In order to confirm this supposition the appropriate ethers† of piloceredine were subjected to cleavage with potassium in liquid ammonia at −60° since identical products[4] should be obtained and indeed this proved to be the case. From the non-phenolic fraction of the ether cleavage of piloceredine methyl ether (Ib) there was isolated 1-*iso*butyl-2-methyl-6-methoxy-1,2,3,4-tetrahydro*iso*quinoline (IIa), 1-*iso*butyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro*iso*quinoline (IIc) and 1-*iso*butyl-2-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydro*iso*quinoline (IIIa). Methylation of the phenolic cleavage products followed by chromatography afforded 1-*iso*butyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro*iso*quinoline (IIc) and 1-*iso*butyl-2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydro*iso*quinoline (IIIb). The identification of these products establishes structure (Ib) for piloceredine methyl ether. In order to prove rigorously the position of the kryptophenolic hydroxyl group in the free alkaloid, a sample of piloceredine ethyl ether (Ic) was treated with potassium in liquid ammonia at −60° and 1-*iso*butyl-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydro*iso*quinoline (IId) was identified among the cleavage products. The isolation of (IId) *ipso facto* locates the free hydroxyl group of piloceredine; thus it follows that this alkaloid possesses the same structure (Ia) as pilocereine and that both possible diastereoisomers occur in the same cactus.

It has already been mentioned above that both alkaloids were optically inactive. With the information at hand it is impossible to say whether pilocereine and

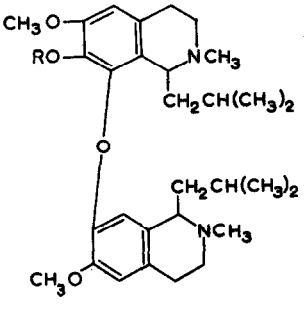
* Established by examination in a spectropolarimeter down to 350 mμ.[1]

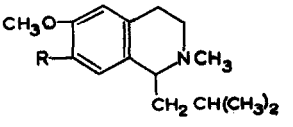
† It has been noted in the case of pilocereine that ether cleavage of the base itself is accompanied by skeletal rearrangement.[1]

[4] The synthesis of the relevant methoxy and ethoxy tetrahydro*iso*quinolines has already been recorded by C. Djerassi, F. X. Markley and R. Ehrlich *J. Org. Chem.* 21, 975 (1956).

piloceredine exist as racemates in the cactus or whether racemization occurred during the isolation procedure. Späth and Kesztler[5] have observed that resolved, synthetic pellotine (IV) racemizes quite readily. In order to determine whether this also applies to pilocereine and piloceredine a variety of unsuccessful resolutions were attempted as outlined in the experimental section.

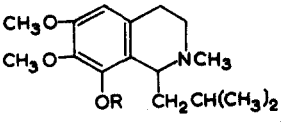
Of the total alkaloids of the cactus *Lophocereus Schottii* approximately 5 per cent is soluble in alkali. The phenolic alkaloids could not be purified but when a distilled portion was methylated with diazomethane and subjected to careful chromatography there was isolated—as the picrate—a small amount of 1-*iso*butyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydro*iso*quinoline (IIc). Consequently, this substance must represent the methyl ether of another alkaloid—now named lophocerine—and we are suggesting structure IIb (1-*iso*butyl-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydro*iso*quinoline) for it, although it is appreciated that the position of the free phenolic group(s) follows only from analogy.* We consider the isolation of lophocerine (IIb) to be of some biogenetic significance since both monomeric (IIb) and dimeric (Ia) fragments are found in the same cactus. It is very likely that pilocereine and piloceredine are produced by phenol coupling and lophocerine (IIb) contains all of the requisite structural features[6] for such a biosynthetic step.

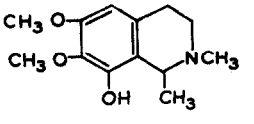




I a R=H
b R=CH_3
c R=C_2H_5
d R=CH_3CO

II a R=H
b R=OH
c R=OCH_3
d R=OC_2H_5





III a R=H
b R=CH_3

IV

* The structure would have been proved rigorously if the ethylation product could have been isolated in pure form but this did not prove to be the case. Our failure is probably due to the fact that only a small portion of the phenolic fraction is represented by lophocerine—most of it being polymeric material—and the expected 1-*iso*butyl-2-methyl-6-methoxy-7-ethoxy-1,2,3,4-tetrahydro*iso*quinoline picrate crystallizes only with difficulty if not completely pure.

[5] E. Späth and F. Kesztler *Ber. Dtsch. Chem. Ges.* **69**, 755 (1936).

[6] R. Robinson *The Structural Relations of Natural Products* p. 85, Oxford University Press (1955); R. H. F. Manske *The Alkaloids* Vol. IV, p. 1, Academic Press, New York (1954).

EXPERIMENTAL*

Isolation of piloceredine (Ia).† A 5·5 kg portion of dried alcoholic extract derived from 36 kg of dry *Lophocereus Schottii* was partitioned between ether and 10 per cent hydrochloric acid, any insoluble material being discarded. The combined acid solutions were basified to pH 9 with ammonium hydroxide which caused the separation of some black gum. After thorough extraction with ether and filtration of any insoluble material the aqueous layer was examined for quaternary salts by addition of ammonium reineckate. The precipitate was converted into the sulfate, chloride and picrate but no evidence for the presence of quaternary salts could be found. Typical experimental procedure is already on record.[7]

The ether layer was washed with 5 per cent sodium hydroxide solution and this was processed separately (see below) for phenolic alkaloids. Concentration of the ether solution to a small volume and chilling in the refrigerator for several days furnished 220 g of substantially pure pilocereine which exhibited m.p. 175–176° after one recrystallization from acetone-hexane.

The oily mother liquor was passed in benzene solution over 7 kg of alumina (deactivated with 210 cm^3 of 10 per cent acetic acid) and the benzene and ether fractions were combined and rechromatographed on 7·5 kg of activated alumina (activity I-II). Development was effected with benzene, benzene-ether and ether and 6 fractions of 2·5 l. each were collected. Fraction 1 (elution with benzene) yielded 13·9 g of a mixture (m.p. 151–161°) of pilocereine and piloceredine which was resolved by repeated chromatography into 3·0 g of pilocereine and 9·5 g of piloceredine. Fractions 2–6 yielded 80·1 g of *piloceredine*, m.p. 161–163°, raised to m.p. 165–166° after recrystallization from acetone. A mixture with pilocereine melted at 153–173° and the two substances could not be interconverted by cross seeding. The infra-red spectra in chloroform and in carbon disulfide were identical, but substantial differences were noted in a nujol infra-red spectrum and in the X-ray diffraction patterns.

Anal. Calcd. for $C_{30}H_{44}N_2O_4$: C, 72·54; H, 8·93; N, 5·64; O, 12·89; 2 methoxyl, 12·49; 2 N—CH_3, 6·05. Found: C, 72·61; H, 8·51; N, 5·59; O, 12·95; methoxyl, 12·30; N—CH_3, 6·89.

Piloceredine diperchlorate. A solution of piloceredine in dilute hydrochloric acid was treated with aqueous ammonium perchlorate and the resulting precipitate was recrystallized from methanol, m.p. 221–222°, depressed to 210–213° upon admixture with pilocereine diperchlorate (m.p. 216–217°). The finger print regions of the respective nujol mull infra-red spectra were different. The analytical sample was dried at 130° and 4 mm for 24 hr.

Anal. Calcd. for $C_{30}H_{44}N_2O_4$ 2 $HClO_4$: C, 51·64; H, 6·64; N, 4·01; O, 27·52; Cl, 10·10. Found: C, 51·31; H, 6·88; N, 4·32; O, 27·25; Cl, 9·99.

Piloceredine acetate (Id). A sample of piloceredine was acetylated with acetic anhydride and pyridine and all volatile material was removed *in vacuo*. The residue was taken up in ether, washed with potassium carbonate solution, dried, evaporated

* Melting points are uncorrected. The microanalyses were carried out by Dr. A. Bernhardt, Mülheim, Germany.

† We are indebted to Parke, Davis and Co., Detroit, Michigan for putting the facilities of their pilot plant at our disposal.

[7] M. Tomita and T. Nakano *J. Pharm. Soc. Japan* 72, 727 (1952).

and chromatographed on deactivated alumina. Elution with benzene and recrystallization from petroleum ether afforded the crystalline acetate, m.p. 133–134°, depressed to 120–128° upon admixture with pilocereine acetate (m.p. 186°); the infra-red spectra of the two samples, measured in carbon disulfide were practically identical.

Anal. Calcd. for $C_{32}H_{46}N_2O_5$: C, 71·34; H, 8·61; N, 5·20; O, 14·85. Found: C, 71·35; H, 8·63; N, 5·34; O, 14·57.

Cleavage of piloceredine methyl ether (Ib). A solution of 4·5 g of piloceredine in 100 cm³ of methanol was treated for 4 days at 0° with distilled ethereal diazomethane (from 10 g of N-nitroso methylurea) and then for a further 6 days with a fresh portion of diazomethane. After evaporation to dryness the residue was recrystallized several times from hexane whereupon the methyl ether (Ib) exhibited m.p. 141–142°. A mixture melting point with pilocereine methyl ether (m.p. 133–134°) was depressed to 124–130°. The infra-red spectra showed marked differences in nujol mull but were essentially identical when measured in chloroform solution.

Anal. Calcd. for $C_{31}H_{46}N_2O_4$: C, 72·90; H, 9·08; N, 5·49; O, 12·53. Found: C, 73·27; H, 8·98; N, 5·40; O, 12·54.

The ether cleavage was accomplished by adding 3·4 g of potassium metal with stirring at −60° to 2·5 g of piloceredine methyl ether (Ib) in 100 cm³ of ether and 500 cm³ of distilled liquid ammonia. After 7 hr ammonium chloride was added to discharge the blue color and the ammonia was allowed to evaporate overnight. The residue was partitioned between ether and 3 per cent sodium hydroxide solution, the organic phase was extracted with dilute hydrochloric acid and basified with ammonium hydroxide and the non-phenolic basic fraction (1·68 g) was re-extracted with ether. Separation was effected by chromatographing this material on 140 g of alumina deactivated with 4·2 cm³ of 10 per cent acetic acid and collecting about 50 fractions.

Fractions 20–25 eluted with benzene furnished—after treatment with picric acid—0·51 g of 1-iso*butyl*-2-*methyl*-6-*methoxy*-1,2,3,4-*tetrahydroisoquinoline* (IIa) *picrate* m.p. 152–153°. Fractions 27–33 (1 : 1 benzene-ether) yielded 0·5 g of 1-iso*butyl*-2-*methyl*-6,7-*dimethoxy*-1,2,3,4-*tetrahydroisoquinoline* (IIc) *picrate* m.p. 183–184° while the ether eluates (fractions 39–43) after conversion to the picrate led to 0·14 g of 1-iso*butyl*-2-*methyl*-6,7-*dimethoxy*-8-*hydroxy*-1,2,3,4-*tetrahydroisoquinoline* (IIIa) *picrate* m.p. 152–153°.

The original 3 per cent sodium hydroxide extracts were made acid with hydrochloric acid, washed with ether (discarded), made alkaline with ammonium hydroxide and extracted with ether. Evaporation of the ether gave 0·72 g of phenolic, basic oil which was methylated in ether-methanol solution for 8 days at 0°. When the methylated phenolic fraction was chromatographed in the above described manner and the various eluates treated with picric acid there was obtained 0·46 g of 1-iso*butyl*-2-*methyl*-6,7-*dimethoxy*-1,2,3,4-*tetrahydroisoquinoline* (IIc) *picrate* m.p. 181–184° and 0·18 g of 1-iso*butyl*-2-*methyl*-6,7,8-*trimethoxy*-1,2,3,4-*tetrahydroisoquinoline* (IIIb) *picrate* m.p. 132–134°. Identification was accomplished in each case by mixture melting point determinations with authentic samples.[1,4,8]

Cleavage of piloceredine ethyl ether (Ic). Piloceredine (6·0 g) was converted into its ethyl ether (6·06 g, m.p. 147–149°) with diazoethane exactly as described above for

[8] C. Djerassi, J. J. Beereboom, S. P. Marfey and S. K. Figdor *J. Amer. Chem. Soc.* 77, 484 (1955).

the diazomethane methylation except that absolute ethanol was used as solvent. The analytical sample obtained from hexane showed m.p. 150–152°, depressed to 133–141° upon admixture with pilocereine ethyl ether (m.p. 147–148°). The infra-red spectra of the two ethers in chloroform solution were identical but differed in the finger print region when measured in nujol mull.

Anal. Calcd. for $C_{32}H_{48}N_2O_4$: C, 73·24; H, 9·22; N, 5·34; O, 12·20; 2 N—CH_3, 5·72. Found: C, 73·32; H, 9·20; N, 5·20; O, 12·43; N—CH_3, 6·09.

The cleavage of 2·5 g of the ethyl ether was conducted as described above for the methyl ether except that the reaction time was extended to 24 hr. From the non-phenolic portion there was isolated after chromatography and conversion to the picrate 0·31 g of 1-iso*butyl*-2-*methyl*-6-*methoxy*-7-*ethoxy*-1,2,3,4-*tetrahydro*isoquinoline (IId) *picrate* m.p. 149–150° which was not depressed on mixture with a synthetic specimen.[4]

Lophocerine (IIb). A portion of the phenolic alkaloids (*c.* 5 per cent of the total alkaloids) was distilled and 0·53 g of material distilling between 150–225° at 0·05 mm was methylated with diazomethane in methanol-ether for 3 days. After washing with alkali and acid, basifying the acid extracts and taking up in ether, evaporation of the dried organic phase gave 0·26 g of oil which was chromatographed on 20 g of alumina deactivated with 0·6 cm^3 of 10 per cent acetic acid. Elution with hexane-benzene (1 : 2), conversion to the picrate and recrystallization from ethanol yielded 80 mg of 1-iso*butyl*-2-*methyl*-6,7-*dimethoxy*-1,2,3,4-*tetrahydro*isoquinoline (IIc) *picrate* m.p. 185–186°, undepressed when mixed with a synthetic sample.[8]

Attempted resolutions. The resolution of pilocereine was attempted with the following reagents without success: D-tartaric acid, L-dibenzoyltartaric acid, D-camphoric acid, D-10-camphorsulfonic acid, D-quinic acid, L-malic acid, N-acetyl-L-leucine and (+)-N-acetyl-threo-β-phenyl-l-serine (kindly provided by Dr. A. Fürst, Hoffmann-LaRoche, Basel).

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