## 286. *Alkaloids of* Physostigma venenosum. *Part I. The Structure of Physovenine.*

By B. Robinson.

Physovenine, together with a new alkaloid and the lower-melting isomorph of physostigmine, has been isolated from the basic extract of *Physostigma venenosum* seeds. A structure (V) is advanced for physovenine upon evidence mainly obtained by comparison of its ultraviolet, infrared, and proton magnetic resonance spectra with those of physostigmine.

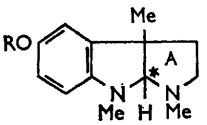
The seeds of *Physostigma venenosum* (Calabar beans) have been known for nearly 100 years to contain the alkaloid physostigmine (eserine), the structure of which is known to be (I; R = MeNH·CO).[1] Apart from this major alkaloid, traces of other bases have been isolated from these seeds and one of these, geneserine, has been shown to be the $N_b$-oxide of physostigmine (II).[2] Information regarding the other minor alkaloids has so far usually been confined to melting point and empirical or molecular formula [3] owing to the small quantities of material available.

It has now been found that partial crystallisation followed by chromatography of the basic residue remaining after the industrial isolation of physostigmine from *Physostigma*

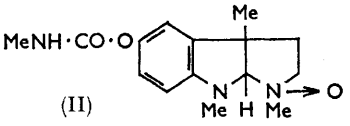
[1] For a recent review, see B. Robinson, *Chem. and Ind.*, 1963, 218.
[2] Polonovski and Nitzberg, *Bull. Soc. chim. France*, 1915, **17**, 244; 1917, **21**, 191; 1918, **23**, 335, 356.
[3] See Henry, "The Plant Alkaloids," J. and A. Churchill Ltd., London, 1949, p. 547.

*venenosum* seeds (these residues were supplied as the mixed salicylates by Burroughs Wellcome and Co.) effects the separation of three alkaloids. One of these is the lower melting isomorph of physostigmine,[4] another is a new alkaloid (now being studied), and the third is physovenine.

Physovenine was first isolated in 1911 by fractional crystallisation of the basic extract of *Physostigma venenosum* seeds.[4] It has m. p. 123°, empirical formula $C_{14}H_{18}N_2O_3$, and




(I)




(II)

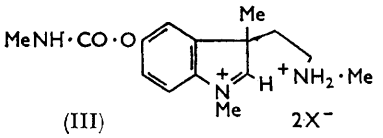
liberates carbon dioxide and assumes a red colour when treated with barium hydroxide.[4] This represented the total information on the alkaloid until the present investigation was undertaken, and had led to the suggestion [4] that physovenine is an intermediate in the conversion of physostigmine (I; R = MeNH·CO) into eseroline (I; R = H).

The above empirical formula, now shown to be the molecular formula, has been confirmed. The infrared spectrum of physovenine indicated the presence of the MeNH·CO·O grouping in the molecule owing to strong, sharp absorption bands at 1751 and 3473 cm.$^{-1}$ [cf. the MeNH·CO·O grouping in physostigmine (I; R = MeNH·CO) and geneserine (II) which give rise to similar absorption at 1752 and 3474 cm.$^{-1}$ (C=O and N–H stretching, respectively)]. Since no absorption other than that mentioned above occurs in the 3600—3100 and 1850—1625 cm.$^{-1}$ regions, the third oxygen atom in physovenine most probably occurs as an ether linkage. Apart from these similarities, the complete infrared spectra of physovenine and physostigmine showed many common features, therefore suggesting that the two alkaloids were structurally closely related, this also being supported by comparison of (*a*) the rotations of the alkaloids {physovenine $[\alpha]_D^{22.5°}$ −92° (in ethanol); physostigmine $[\alpha]_D$ −75·8° and −120° (in chloroform and benzene, respectively) [5]} and (*b*) their ultraviolet absorption spectra [physovenine $\lambda_{max.}$ 252 and 310 mμ (ε 13,200 and 3300, respectively); physostigmine $\lambda_{max.}$ 253 and 310·5 mμ (ε 12,100 and 2800, respectively)]. These u.v. spectral measurements not only showed that physovenine contains an indoline nucleus, but also that the MeNH·CO·O grouping is substituted at the 5-position of that nucleus as in physostigmine (it is known that the position of a benz-substituent on an indoline nucleus markedly affects the u.v. absorption; cf. ref. 6), this also being supported by biogenetic considerations and by the i.r. spectrum of physovenine, which contains weak absorption bands at 1183, 1103, 1075, and 1063 cm.$^{-1}$ characteristic of a 1,2,4-trisubstituted benzene ring.[7]

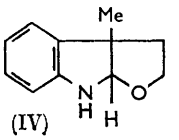
However, unlike the ultraviolet spectrum of physostigmine, which in dilute acid undergoes a hypsochromic shift of about 10 mμ because of protonation of $N_b$ in the Ph·$N_a$–C–$N_b$ system,[8] that of physovenine in 1·5N-ethanolic hydrochloric acid shows no such shift but has absorption characteristic of a combination of 3*H*-indolinium cation and indoline chromophores. This therefore eliminated the possibility that physovenine contains a Ph·N–C–N system.[1,8] In 11N-hydrochloric acid, however, both physovenine and physostigmine exhibit u.v. absorptions characteristic of a pure 3*H*-indolinium cation. In physostigmine this is due to cleavage of the $N_b$–C* bond with the formation of structure (III),[9] and since physovenine does not contain a Ph·N–C–N system but does contain an ether linkage, it can be ascribed in this case to similar cleavage of a Ph·N–C–O system (cf.

[4] Salway, *J.*, 1911, **99**, 2148.
[5] Ref. 3, p. 540.
[6] Chalmers, Openshaw, and G. F. Smith, *J.*, 1957, 1115.
[7] Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen and Co. Ltd., London, 1958, p. 65.
[8] Hodson and G. F. Smith, *J.*, 1957, 1877.
[9] Jackson and A. E. Smith, personal communication.

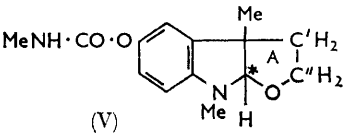
picraline,[10] deacetylpicraline,[10] ψ-akuammigine,[11,12] and *O*-methylakuammine [11]). The difference in behaviour of this system in physovenine with that reported [13] for structure (IV),[14] which shows indoline absorption in ethanol, benzenoid absorption in dilute acid, and a mixture of benzenoid and 3*H*-indolinium cation absorption in concentrated acid, is probably due to the absence from (IV) of MeNH·CO·O at the 5-position of the indoline nucleus (cf. ref. 11).



(III)



(IV)



(V)

Structure (V) can be postulated for physovenine from these data and biogenetic considerations involving a scheme similar to that postulated [15] for the biogenesis of physostigmine, except that, in the case of physovenine, transformation of the amino-ethyl side-chain to a hydroxyethyl side-chain [probably by dehydrogenation (−2H) of the amine to the imine, followed by hydrolysis with subsequent reduction (+2H) to the alcohol] occurs before the formation of ring A.

This structure was subsequently confirmed by the following complete analyses and comparison of the p.m.r. spectra of physostigmine and physovenine.

In the spectrum of physostigmine, the C–Me, aliphatic N–Me, aromatic N–Me, and C*–H protons give rise to sharp singlets at τ 8·53, 7·36, 7·00, and 5·77 with intensities 3, 3, 3, and 1, respectively. The three aromatic protons form an ABX system with a multiplet between τ 3·19 and 3·01 of intensity 2 and a multiplet between τ 3·65 and 3·49 of intensity 1. The urethane N–H proton gives a broad band between τ 4·71 and 4·08 of intensity 1 and the methyl group attached to this nitrogen atom gives a doublet ($J$ = 4·8 c./sec.) at τ 7·16 and 7·09, which is superimposed upon a multiplet due to the $N_b$–$CH_2$ protons, the total intensity of this series of bands between τ 7·30 and 7·01 being 5. The remaining methylene protons, which apparently form an ABXY system with the $N_b$–$CH_2$ protons (probably owing to the rigidity of ring A), give rise to a multiplet between τ 8·09 and 7·81 of intensity 2.

The spectrum of physovenine shows singlets at τ 8·51, 7·02, and 4·83 of intensities 3, 3, and 1 indicating the C–Me, aromatic N–Me, and C*–H protons, respectively, and shows no signal due to protons of an aliphatic N–Me group [the absence of such a group in physovenine is also apparent from the i.r. spectrum, that of physostigmine having an absorption band at 2791m cm.$^{-1}$ characteristic of an aliphatic N–Me group,[16] no such band appearing in the spectrum of physovenine]. Although the two high-field absorption bands are at approximately the same τ values with corresponding bands in the spectrum of physostigmine, the τ value of the C*–H proton has experienced a down-field shift of 0·94 compared with the corresponding proton in physostigmine, as would be expected of a proton attached to a carbon atom flanked by an oxygen and a nitrogen atom instead of two nitrogen atoms. The three aromatic protons again form an ABX system giving rise to a multiplet between τ 3·22 and 3·03 of intensity 2 and a multiplet between τ 3·73 and 3·58 of intensity 1. The urethane N–H proton gives a broad band between τ 4·91 and 4·33 of intensity 1 and the methyl group attached to this nitrogen gives a doublet ($J$ = 4·8 c./sec.) at τ 7·13 and 7·05 of intensity 3, these being of the same order as the corresponding

[10] Britten, G. F. Smith, and Spiteller, *Chem. and Ind.*, 1963, 1492.
[11] Joule and G. F. Smith, *J.*, 1962, 312.
[12] Britten, Edwards, Joule, G. F. Smith, and Spiteller, *Chem. and Ind.*, 1963, 1120; Olivier, Lévy, Le Men, Janot, Djerassi, Budzikiewicz, Wilson, and Durham, *Bull. Soc. chim. France*, 1963, 646.
[13] Bardsley, M.Sc. Thesis, Manchester, 1961.
[14] Hoshino and Shimodaira, *Annalen*, 1935, **520**, 19.
[15] Sir R. Robinson, " The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 103.
[16] Hill and Meakins, *J.*, 1958, 760.

absorption bands in the spectrum of physostigmine. The remaining four protons in physovenine, the methylene protons, form an ABXY system as in physostigmine which gives rise to two mutiplets, each of intensity 2. That due to the C'$H_2$ protons is between $\tau$ 8·04 and 7·74 and that due to the C''$H_2$ protons being between $\tau$ 6·74 and 5·76, this latter multiplet having undergone a down-field shift compared with the corresponding multiplet in the spectrum of physostigmine, as expected of methylene protons attached to a carbon atom flanked by a carbon and an oxygen atom instead of by a carbon and a nitrogen atom.

## Experimental

Melting points were determined on a Kofler block and are uncorrected. The optical rotation was measured on a Bellingham and Stanley polarimeter. Ultraviolet spectra were measured in ethanolic solution (unless otherwise stated) on a Unicam S.P. 700 spectrophotometer. Infrared spectra were recorded in carbon tetrachloride on a Unicam S.P. 100 spectrophotometer fitted with a grating attachment. Proton magnetic resonance spectra were determined in deuterochloroform solution on an A.E.I. spectrometer R.S.II operating at 60 Mc./sec.; tetramethylsilane being used as internal standard and intensities being measured by using a planimeter.

*Separation of the Alkaloids and Isolation of Physovenine.*—The salicylate residues (80 g.) were partitioned between 2N-hydrochloric acid (1 l.) and ethyl acetate (1·5 l.). The dark brown acidic layer was separated off and the ethyl acetate layer further extracted with 2N-hydrochloric acid (500 ml.) before being discarded. The combined acidic layers were washed with ethyl acetate (2 × 500 ml.) and then carefully basified with saturated aqueous sodium carbonate solution followed by extraction with ether (6 × 500 ml.). After being dried ($MgSO_4$) and evaporated, the combined ether extracts gave a dark brown gum (38 g.). On addition of ether (250 ml.) to this gum, a little insoluble solid remained; this was filtered off, washed with ether, and air-dried. Two crystallisations from ethanol afforded fine white needles (0·44 g.), m. p. 216—218° (slight decomp.) (Found: C, 59·7, 60·0; H, 7·05, 6·75; N, 17·4, 17·6. $C_{16}H_{22}N_4O_3$ requires C, 60·35; H, 6·95; N, 17·6%).

The ether-soluble fraction of the gum was chromatographed on alumina (grade H). With ether (2·5 l.) as solvent a yellow band was eluted; this gave a brown oil which would not crystallise. It was rechromatographed on alumina (grade H) and the yellow band collected in two equal fractions, the first giving a pale brown oil which completely crystallised on trituration with ether–light petroleum (b. p. 60—80%). After two recrystallisations from ether, white plates (0·31 g.), m. p. 124—125° (cf. physovenine, m. p. 123°),[4] were obtained [Found: C, 64·2; H, 6·95; N, 10·8% (cf. physovenine, Found: 63·8, 63·8; H, 7·3, 7·0; N, 10·6%);[4] *M*(Rast), 252; *M* (mass spectra), 262. Calc. for $C_{14}H_{18}N_2O_3$: C, 64·1; H, 6·9; N, 10·7%; *M*, 262], $\lambda_{max.}$ 233, 239, and 284 mμ (ε 6600, 6100, and 5400, respectively, in 11N-hydrochloric acid). The second fraction after removal of the ether, remained as a pale brown gum which was not examined further.

Elution of the main column with ether (15 l.) was continued; removal of the solvent from the yellow eluate gave a brown gum (14·6 g.) which partially crystallised on trituration with ether and cooling at 5° for 24 hr. The solid, after one crystallisation from ether–light petroleum, was obtained as white prisms (4·3 g.), m. p. 85—86° (cf. the lower-melting isomorph of physostigmine, m. p. 86—87°).[4] The infrared spectrum of this compound was identical with that of a sample of physostigmine, m. p. 101° (supplied by Burroughs Wellcome and Co.).

Elution was then continued with chloroform (6 l.) and a red band was eluted. Removal of the solvent from this gave a brown gum which partially crystallised on trituration with ether. Crystallisation of the solid from ethanol gave fine white needles (0·13 g.), m. p. 216—218° (slight decomp.) which showed no depression of melting point with and which had identical infrared spectrum to that of the alkaloid, m. p. 216—218° (slight decomp.) obtained before chromatography. Further elution of the column with chloroform, chloroform–methanol, and methanol gave only gums and tars which did not yield crystalline compounds.

This work was carried out during the tenure of an Imperial Chemical Industries Post-doctoral Research Fellowship. I thank Burroughs Wellcome and Co. for the generous supply of the mixed base salicylate residue and for the gift of authentic physostigmine.

Department of Chemistry, The University, Nottingham. [*Received, September 30th*, 1963.]