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ALKALOIDS OF *PIPER LONGUM* LINN—I

STRUCTURE AND SYNTHESIS OF PIPERLONGUMINE AND PIPERLONGUMININE

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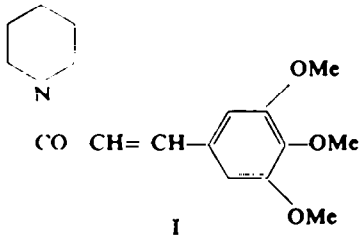
Abstract—From the root of *Piper longum* L. two new alkaloids, piperlongumine and piperlonguminine have been isolated. Piperlongumine and piperlonguminine are shown to be N-(3,4,5-trimethoxy-cinnamoyl)-Δ^5-piperidin-2-one and isobutylamide of piperic acid respectively.

Piper longum, a climbing plant grows in abundance in the tropical regions of India, known as "Pippali" it is often used as an effective drug[1] in the treatment of asthma and chronic bronchitis in the Ayurvedic system of medicine.

Earlier investigations[2–6] with other *Piper* species, have led to the isolation of several alkaloids, the structures of which reflect that they all contain a piperidine moiety. The alkaloid amides isolated from the roots of *P. longum*, however, deviate from this generality.

Recently, the occurrence of an alkaloid amide, piplartine (I) was observed[7,8] in the stem-bark of *Piper longum*. From the root of the same species we isolated two new alkaloids, piperlongumine[9] and piperlonguminine[10] besides piperine and a minor liquid base which is a colourless oil and is yet to be characterized.



I

Piplartine

The isolation, structure and synthesis of piperlongumine and piperlonguminine are elaborated in the present communication.

Piperlongumine*, was isolated from the petrol extract of the dried roots. It was

* The Chemistry of Piperlongumine was presented by A. C. in the *IUPAC Symposium on The Chemistry of Natural Products*, Kyoto, Japan (1964).

[1] Kirtikar and Basu, *Indian Medicinal Plants* Vol. III; p. 2128. Basu, Allahabad, India (1933).
[2] F. S. Spring and J. Stark, *J. Chem. Soc.* 1177 (1950).
[3] F. Otto and F. Eichler, *Ber. Dtsch. Chem. Ges.* **55**, 2653 (1922).
[4] W. R. Dunstan and H. Garnett, *J. Chem. Soc.* **67**, 95, 100 (1895).
[5] K. Peimann, *Arch. Pharm.* **234**, 204 (1896).
[6] J. Stenhouse, *Pharm. J. Trans.* **14**, 36 (1855).
[7] C. K. Atal and S. S. Banga, *Ind. J. Pharm.* **24**, 105 (1962).
[8] C. K. Atal and S. S. Banga, *Curr. Sci.* **32**, 354 (1963).
[9] A. Chatterjee and C. P. Dutta, *Sci. and Cult* **29**, 568 (1963).
[10] A. Chatterjee and C. P. Dutta, *Tetrahedron Letters* 1797 (1966).

shown to have the formula $C_{14}H_{10}NO_3(OCH_3)_3$ by mass spectrometric determination of the mol. wt. 317, and analysis of the alkaloid and its various derivatives. It was found to be a monoacidic weak base (pK_a, 4·50) and it failed to form any salt of the type B·HX or B·CH_3I. The three absorption maxima in the UV spectrum (Fig. 1) of piperlongumine in neutral ethanol quenched to one λ_{max}^{EtOH,H^+} 328 mμ, (log ε, 4·26) in presence of acid. The UV spectrum of the alkaloid in ethanolic alkali $\lambda_{max}^{EtOH,OH^-}$ 232 (log ε, 4·37) and 304 mμ (log ε, 4·28) was, however, found to be comparable with that of 3,4,5-trimethoxycinnamic acid. The IR spectrum of the alkaloid showed strong bands at 5·98 and 6·20 μ for amide carbonyl and unsaturation.

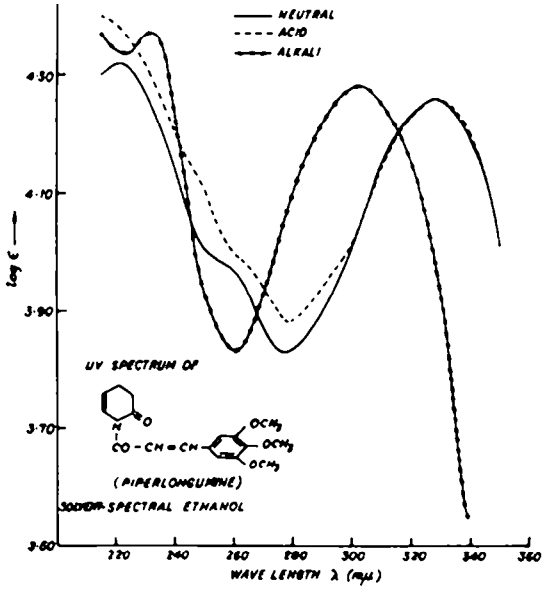
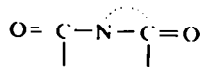


FIG. 1

Piperlongumine gave a positive test with tetranitromethane and furnished on catalytic reduction a tetrahydro derivative, $C_{17}H_{23}NO_5$, m.p. 84° (M^+, 321). The IR spectrum of tetrahydropiperlongumine lacked the strong band at 6·20 μ for ethylenic unsaturation and in its NMR spectrum the four olefinic proton signals (4·00, 3·82, 2·35 and 2·10 τ), present in the parent alkaloid, were found missing. The IR spectrum of tetrahydropiperlongumine showed twin amide carbonyl peaks at 5·90 and 5·95 μ indicating the presence of two amides or rather an imide (piperlongumine contains one nitrogen atom) linkage in its molecule.

In conformity with the imide structure of the alkaloid hydrolysis of piperlongumine in ethanolic alkali gave 3,4,5-trimethoxy-cinnamic acid, $C_{12}H_{14}O_5$, m.p. 124–126°, and a nitrogen containing acid, designated as piperlongumic acid, $C_{17}H_{21}NO_6$, m.p. 174°, the latter having the same number of carbon and nitrogen atoms as that of the parent alkaloid. The UV absorption piperlongumic acid was found to be strikingly similar to that of 3,4,5-trimethoxycinnamic acid and its IR spectrum was essentially the same as that of the original alkaloid except that it showed new absorption bands at 3·0. 3·25, 6·10 μ for monosubstituted amide and a band at 5·90 μ for a

carboxyl group. The formation of piperlongumic acid thus involved a fission of a cyclic imide of the type



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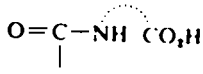
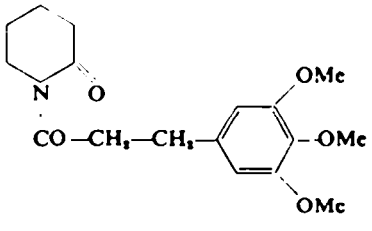


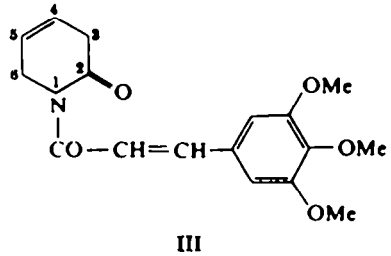
without loss of any fragment. Catalytic hydrogenation of piperlongumic acid furnished tetrahydropiperlongumic acid, $C_{17}H_{25}NO_6$, m.p. 114° which exhibited IR bands at 2·90 (—NH), 5·90 (—COOH) and 6·08 μ (—N—C=O). The fact that the IR band for the carboxyl group in piperlongumic acid remained unaltered but its amide carbonyl band was shifted to lower wavelength after catalytic reduction clearly indicated that its amide linkage and not the free carboxyl group was in conjugation with a double bond.

The hydrolysis of tetrahydropiperlongumine, unlike piperlongumine, furnished an acid (3,4,5-trimethoxyphenylpropionic acid, m.p. 102°) and a liquid base, C_5H_9NO, b.p. 105–110°/2–4 mm. The molecular formula of the liquid base, and its IR spectral bands at 2·95 μ (—NH) and 5·80 μ (6-membered lactam) suggested its α-piperidone structure which was conclusively verified by its conversion to δ-amino-n-valeric acid hydrochloride, m.p. 84° by refluxing with concentrated HCl and finally by its direct comparison (TLC and IR) with synthetic α-piperidone.

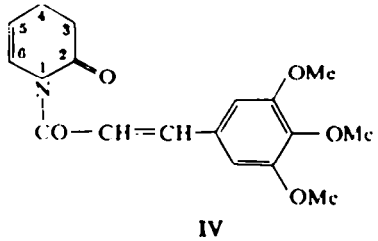
The chemical and spectral data cited so far could be summarized in terms of the expression II for tetrahydropiperlongumine and the alternatives III or IV for piperlongumine.



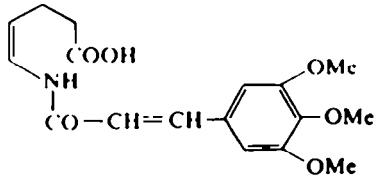
II
Tetrahydropiperlongumine



III



IV



V
Piperlongumic acid

In order to decide whether III ro IV represents the correct structure of piperlongumine it was necessary to determine the position of the double bond in the piperidone ring.

This was possible by ozonolysis of the piperlongumic acid which furnished 3,4,5-trimethoxybenzaldehyde and succinaldehydic acid (identified as its DNPH derivative). The isolation of succinaldehydic acid provided definitive evidence for the location of the double bond at C-5–C-6 in the piperidone ring. It follows therefore that the structure IV must be assigned to piperlongumine and V to piperlongumic acid. The expression IV for piperlongumine thus derived is in complete consonance with its NMR spectrum as evidenced below.

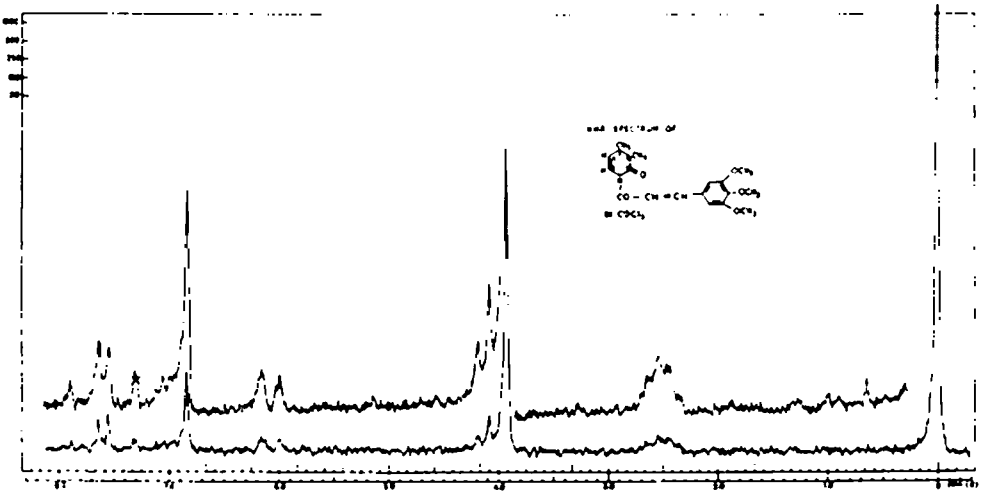
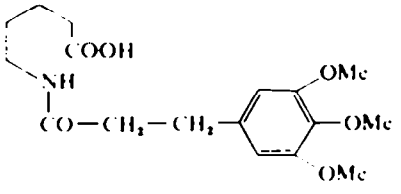


FIG. 2

The 60 Mc NMR spectrum (Fig. 2) of the base exhibited a tall 9 proton signal at 6·10 τ for three aromatic methoxyls and a multiplet around 7·50 τ assignable to the 4 protons attached to C-3 and C-4 carbon atoms of the nitrogenous moiety. A triplet at 4·00 τ and a doublet at 2·35 τ are due to the two olefinic protons at C-5 and C-6 of the same moiety while the peaks at 3·82 τ and 2·10 τ are associated with the two olefinic protons of the cinnamic acid residue. Finally a sharp singlet at 3·10 τ is due to the two aromatic protons of the benzene ring.

Apart from the NMR evidence detailed above, the postulated structure of piperlongumine was also supported by an unambiguous synthesis of tetrahydropiperlongumic acid (VI). Condensation of 3,4,5-trimethoxycinnamoyl chloride with piperidine[11] yielded N-(3,4,5-trimethoxycinnamoyl) piperidine, m.p. 101–102°. The latter upon catalytic reduction furnished the corresponding dihydroderivative $C_{17}H_{25}NO_4$, m.p. 76–78°, which on oxidation with potassium permanganate yielded



VI

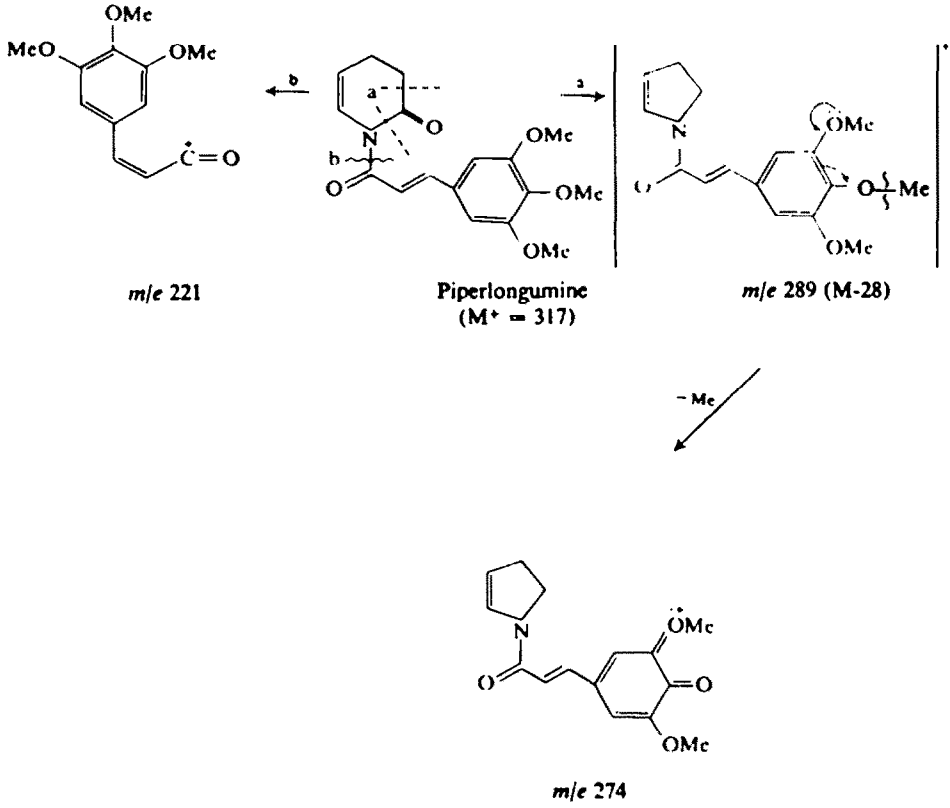
[11] S. C. Schotten, *Ber. Disch. Chem. Ges.* **17**, 2545 (1880).

an acid (VI), $C_{17}H_{25}NO_6$, m.p. 114°, identical in all respects (m.m.p., IR spectra, X-ray powder data) with tetrahydropiperlongumic acid.

The synthetic intermediate, N-(3,4,5-trimethoxycinnamoyl) piperidine, should be structurally identical with piplartine (I) isolated from the stem bark of the same botanical species,[7,8] but the physical constants of the synthetic product are completely different from the values reported for the natural piplartine. This fact coupled with the close similarity between the physical constants of piperlongumine and piplartine suggest that piplartine is probably piperlongumine* thereby suggesting the untenability of the structure advanced for piplartine.

The mass spectral analysis of piperlongumine and its tetrahydro derivative are in complete agreement with the structure advanced for piperlongumine. The important peaks recorded in the spectra and their probable mode of formation from the parent molecules are shown in schemes I and II.

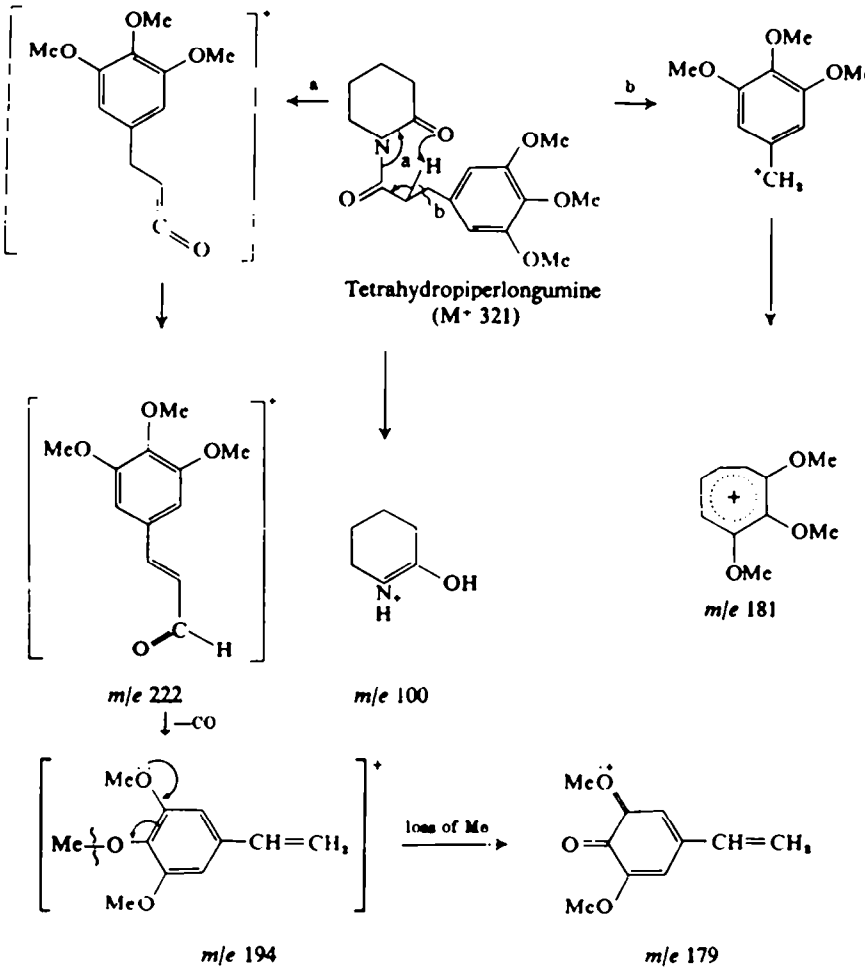
Scheme I. Mass spectral Fragmentation Pattern of Piperlongumine.



The second new alkaloid, piperlonguminine, is a minor constituent of the roots of *P. longum*. Its molecular formula, $C_{16}H_{19}NO_3$, based on analysis was in agreement with the mass spectrometrically derived mol. wt, 273. Piperlonguminine did not give

* Dr. C. K. Atal has reported in a personal communication that piplartine is identical with piperlongumine.

Scheme II. Mass spectral fragmentation pattern of Tetrahydropiperlongumine.



crystalline salt and its neutral character was revealed from its insolubility in acid and alkali. The alkaloid was found to contain an active hydrogen, associated with a monosubstituted amide and a methylenedioxy group. Kuhn-Roth determination corresponded to the presence of more than one C-methyl group but its NMR spectrum (six proton doublets around 8·95 τ) indicated that it contains an isopropyl side chain.

The chromophoric similarity of piperlonguminine with its congener alkaloid, piperine was revealed from their comparable UV spectra (Fig. 3). The IR spectrum exhibited bands at 3·05, 3·25 and 6·10 μ typical of monosubstituted $\alpha\beta$-unsaturated carboxyamide grouping.[18] The intensity of the —C=C— stretching vibration at 6·20 μ suggested an extended conjugation, an additional evidence for which was

[18] L. Crombie, *J. Chem. Soc.* 995, 997, 1007 (1955).

secured from a comparatively low carbonyl frequency at 6·10 μ for the amide carbonyl. The absorption peaks at 7·29 and 7·34 μ, 10·80 μ and 10·10 μ were attributed to isopropyl,[13] methylenedioxy and *trans* configuration of an olefinic double bond[12] respectively.

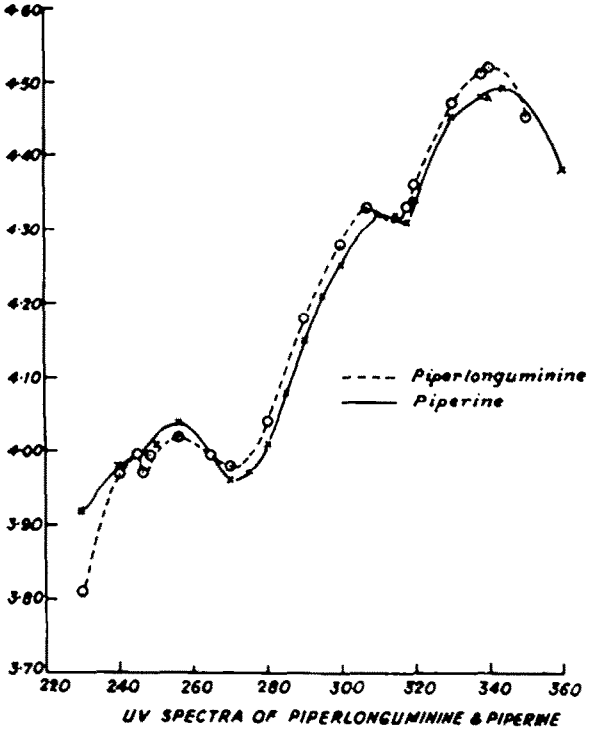
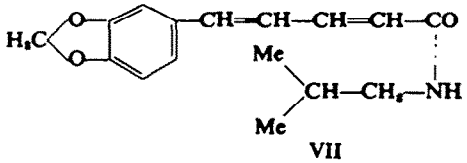


FIG. 3

Piperlonguminine gave a faint yellow colouration with tetranitromethane and decolourized bromine. On catalytic hydrogenation it furnished a tetrahydro derivative, $C_{16}H_{23}NO_3$, m.p. 66° which exhibited a typical methylenedioxy benzene[14] UV spectrum.

In order to ascertain the amide nature of piperlonguminine and to secure knowledge about its basic moiety it was subjected to acid hydrolysis in a sealed tube. The product was a complex mixture from which only isobutylamine hydrochloride, could be isolated and identified. Ozonolysis of the parent alkaloid produced piperonal, (characterized as its DNPH derivative,) and on treatment of the alkaloid with 40% ethanolic alkali piperic acid was obtained. The very isolation of piperic acid and isobutylamine by the alkali and acid hydrolysis respectively establishes that piperlunguminine is an isobutylamide of piperic acid (VII).



VII

[13] K. R. Varma, M. L. Maheswari and S. C. Bhattacharyya, *Tetrahedron* **21**, 115 (1965).
[14] W. J. Genster and C. M. Camour, *J. Org. Chem.* **18**, 9 (1933).

Supportive evidence in favour of VII for piperlonguminine is presented from its NMR spectral (Fig. 4) study. In the low field region the spectrum exhibits signals for methylenedioxy group (2H, 4·10 τ), aromatic protons (3H, 3·20–3·04 τ) and olefinic protons (4H, 3·99, 3·68, 3·25 and 2·50 τ). All these signals appear essentially at the same positions as are observed in the NMR spectrum[15] of piperine thereby affirming the presence of piperic acid system in the parent alkaloid. The six proton methyl doublet ($J = 6$ c/s) around 8·95 τ coupled with an one proton multiplet around 7·90 τ reveals the occurrence of an isopropyl side chain in its molecule. The two proton signal centered at 6·55 τ is attributed to the methylene protons adjacent to the secondary nitrogen atom.

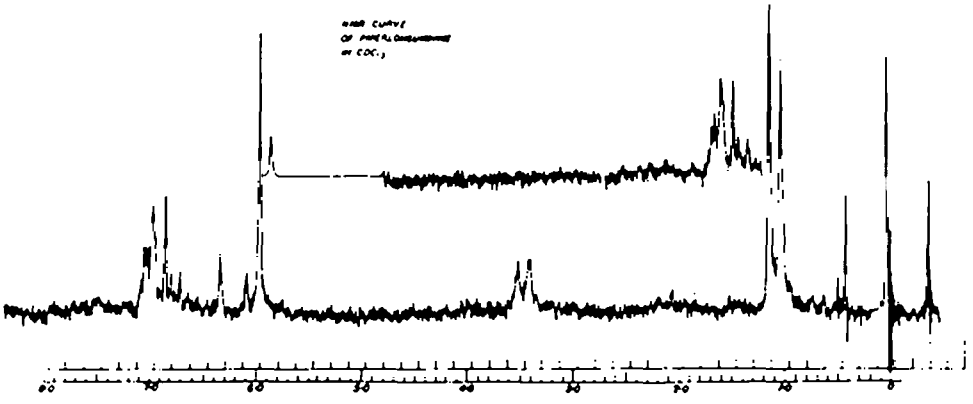
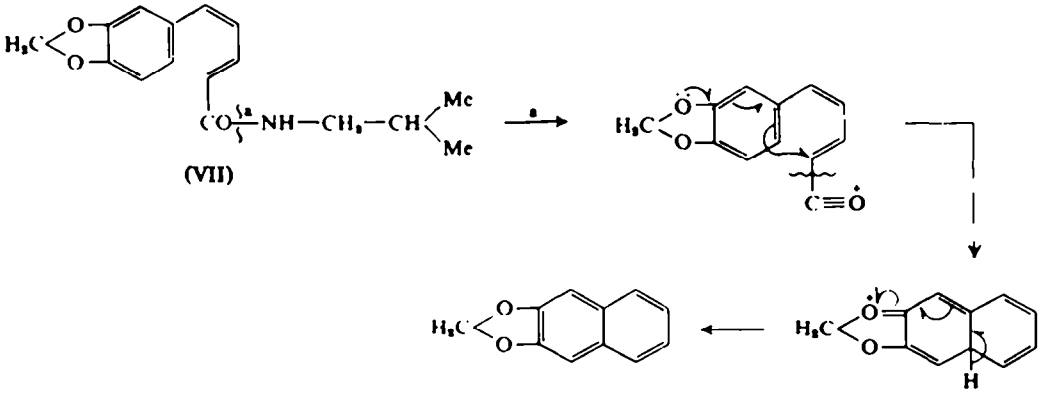


FIG. 4

Confirmatory evidence for the proposed structure VII of piperlonguminine has been available from mass spectral analyses of the alkaloid and its tetrahydro derivative. The mass spectrum of piperlonguminine in addition to the molecular ion peak at *m/e* 273 exhibits characteristic peaks at *m/e*, 201, 173 and 172, the genesis of which can be rationalized as follows:



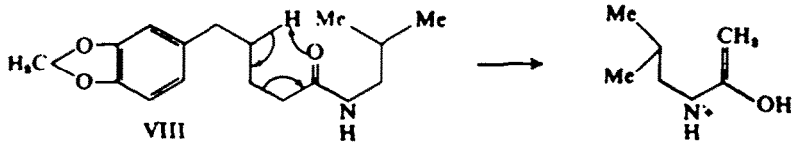
(VII)

m/e 172 *m/e* 173

[15] N. S. Bhacca, L. F. Johnson and J. N. Shoolery, *NMR Spectra Catalog* Varian Associates, U.S.A. (1962).

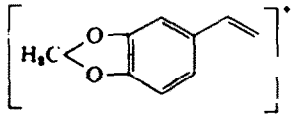
The tetrahydro compound VIII shows the molecular ion peak at *m/e* 277 and the peaks at *m/e* 205, 204 and 115 corresponding to the loss of 72, 73 and 162 mass units respectively.

The ion of mass 204 evidently arises by loss of isobutylamine while McLafferty type of rearrangement gives rise to the ion mass 115.

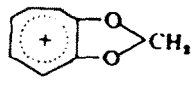


VIII *m/e* 115

Tetrahydropiperlonguminine also exhibits two other important peaks at *m/e* 148 and 135 which are attributed to the ions *a* and *b* respectively.



a m/e 148



b m/e 135

The peak at *m/e* 135 which is common to both the parent alkaloid as well as its tetrahydro derivative is in agreement with the presence of a piperonylidene or a piperonyl group.[16] The ion of mass 115, though less intense, occurs also in the spectrum of piperlonguminine but it can not be said with certainty whether they represent the same ionic species.

The structure VII was finally established by an unequivocal synthesis of piperlonguminine achieved by condensation of piperic acid chloride with isobutylamine in dry benzene.

EXPERIMENTAL

M.ps and b.ps are uncorrected. UV spectra were measured in a Beckmann model DU instrument in 95% EtOH. IR spectra were taken on a Perkin–Elmer Infracord as mulls in nujol. The alumina used for chromatography is a Brockmann's (S. Merk) grade. Samples were dried at constant temperature at 2–4 mm press over P_2O_5.

Isolation of Piperlongumine, piperlonguminine and piperlongine. Air dried finely pulverized roots (1 kg) were extracted with petrol (b.p. 60–80°) for 40 hr. The extract on cooling yielded a partially crystalline residue (5 g; 0·5%) which was triturated with cold ether until the washings were colourless. This yielded virtually pure piperlongumine (2·5 g; 0·25%), m.p. 120–122° which was recrystallized from EtOH–ether (3:1) giving white shining needles, m.p. 124°, $[\alpha]_D^{30} \pm 0$ (dry EtOH), λ_{max}^{EtOH} 220 mμ (log ε, 4·99), and 328 mμ (log ε, 4·50), $\lambda_{max}^{EtOH/0\cdot01N}$ 328 mμ (log ε, 4·28). (Found: C, 64·28; H, 6·05; N, 4·41; OMe, 29·45. $C_{14}H_{19}O_5N(OMe)_2$ requires: C, 64·35; H, 5·99; N, 4·41; OMe, 29·33%.)

Piperlonguminine (IV). The mother liquor was concentrated (50 ml) and together with the washings was chromatographed over alumina using benzene, benzene–chf and pure chf respectively as eluents. The eluates were collected in fractions of 50 ml each, earlier fractions of pure benzene furnished β-sitosterol, m.p. 137°, while the later fractions of benzene–chf (3:1) afforded piperine, m.p. 128°

[16] B. Willhalm, A. F. Thomas and F. Gautschi, *Tetrahedron* **20**, 1185 (1964).

and a new alkaloid piperlonguminine, m.p. 166–68° (0·022%). (Found: C, 69·49; H, 6·56; N, 5·40. $C_{17}H_{19}O_5N$ requires: C, 70·32; H, 6·98; N, 5·12%.) λ_{max}^{EtOH} 245, 256, 307 and 340 mμ (log ε, 3·90; 4·02, 4·33 and 4·52).

Piperlongine. Pure chf eluates yielded another new base, *piperlongine*, B.P. 110–15°/2–4 mm press (0·02%).

Tetrahydropiperlongumine (II). Piperlongumine (0·5 g) in glacial AcOH (50 ml) was hydrogenated using Adams PtO_2 catalyst (0·1 g) at ordinary temp and press. Absorption of H was complete after 6 hr during which two moles H were absorbed. The catalyst was filtered off. Evaporation of the solvent under reduced press yielded a syrupy liquid which was taken up in ether. The ether extract was washed first with $NaHCO_3$aq, then with water and finally dried over Na_2SO_4. Removal of the solvent furnished *tetrahydropiperlongumine* (0·40 g which gave colourless prisms, m.p. 83–84°, from dry ether. (Found: C, 63·67; H, 7·18; N, 4·66; OMe, 29·01; Mol wt 321 (mass spectrometry) $C_{14}H_{14}O_3N$ $(OMe)_3$ requires; C, 63·55; H, 6·54; N, 4·35; OMe, 28·97%, and mol wt 321.)

Ozonolysis of piperlongumine (IV) *and formation of 3,4,5-trimethoxybenzaldehyde*. A stream of ozonized oxygen (ca. 5%) was slowly bubbled through a soln of piperlongumine (0·5 g) in AcOEt (75 ml) kept at 0–5° till no further O_3 was absorbed. Mg turnings (0·2g) and 50% aqueous AcOH (10 ml) were then added and the mixture was kept overnight at room temp. The solvent was removed under diminished press and the residue was thoroughly extracted with chf (4 × 50 ml). The chf extract was washed with water and dried. The oil, obtained after the removal of the solvent was distilled at 160–165°/10 mm yielding 3,4,5-trimethoxybenzaldehyde (0·20 g) crystallized in needles from water, m.p. 78° (identical with an authentic specimen).

It formed well defined DNPH derivative, m.p. 238–240° (AcOEt) with an alcoholic soln (5%) of 2:4-dinitrophenylhydrazine. (Found: N, 14·92. Calc. for $C_{14}H_{14}O_7N_4$. N, 14·89%.)

Alkaline hydrolysis of piperlongumine (IV) *to 3,4,5-trimethoxycinnamic acid and piperlongumic acid* (V). A mixture of piperlongumine (3 g) and KOH (5 g) in EtOH (100 ml) was heated under reflux for 6 hr, then diluted with water (50 ml), the solvent completely removed under reduced press and extracted with chf (500 ml) (*fraction-A*). The aqueous alkaline soln was acidified to pH 5 and extracted with ether (500 ml). The aqueous layer (*fraction-B*) was examined separately. The ethereal extract was washed free from mineral acid (congo red), dried over Na_2SO_4. Evaporation of the solvent yielded 3,4,5-trimethoxycinnamic acid (1·50 g), crystallized from water in fine needles, m.p. 125–126°. The acid was found to be identical with a synthetic sample of 3,4,5-trimethoxycinnamic acid by their mixed m.p., UV and IR spectra. (Found: C, 60·92; H, 5·91; OMe, 38·77; Equiv wt 245. Calc. for $C_9H_8O_2$. (OMe): C, 60·50; H, 5·88; OMe, 39·07% and Equiv wt, 238.)

The aqueous extract (*fraction-B*) was refrigerated for 24 hr. and the crystalline ppt (0·75 g) collected, m.p. 166–170°. The ppt was crystallized from aqueous MeOH in fine flakes, m.p. 172–174°. λ_{max}^{EtOH} 231 and 299 mμ (log ε, 4·42 and 4·33). (Found: C, 61·22; H, 6·45; N, 4·20; OMe, 28·01. $C_{14}H_{18}O_5N$. $(OMe)_3$ requires: C, 60·89; H, 6·26; N, 4·19; OMe, 27·76%.)

Ozonolysis of piperlongumic acid (V) *and formation of 3,4,5-trimethoxybenzaldehyde and succinaldehydic acid*. A stream of ozonized oxygen (ca. 5%) was slowly bubbled through a soln of piperlongumic acid (0·6 g) in glacial AcOH (50 ml) kept at 0–5° till no further O_3 was absorbed. The ozonide thus formed was decomposed by adding Mg turnings (0·5 g) and water (10 ml). The pH of the soln was then raised to 8 by the addition of Na_2CO_3 and the soln extracted with chf (3 × 100 ml). The aqueous soln (*fraction-C*) was kept aside. The chf extract was washed with water, dried and distilled to yield 3,4,5-trimethoxybenzaldehyde, m.p. 76–78°, identical with the authentic specimen.

To the aqueous soln (*fraction-C*) acidified with dil. H_2SO_4 an aqueous soln of 2,4-dinitrophenylhydrazine (5%) was added when a turbidity appeared. The mixture was kept undisturbed when the turbidity increased. It was then extracted with benzene, washed, dried and concentrated (10 ml). The concentrate was chromatographed over silica gel using benzene and benzene–AcOEt (1:1) as eluents. The benzene–AcOEt eluents furnished an orange red DNPH derivative of succinaldehydic acid, (0·925 g), crystallized from MeOH in fine needles, m.p. 202–204° and proved to be identical with DNPH derivative of succinaldehydic acid by mixture m.p. determination with an authentic sample. (Found: C, 41·24; H, 3·30; N, 19·86. Calc. for $C_{10}H_8N_4O$: C, 41·28; H, 3·20; N, 19·92%.)

Tetrahydropiperlongumic acid (VI). Piperlongumic acid (0·4 g) in abs EtOH (40 ml) was hydrogenated in presence of Adams catalyst (0·05 g) and after 2 hr the uptake of H ceased. The filtrate yielded tetrahydropiperlongumic acid (0·38 g), which was crystallized from AcOEt–ether (1:3)in. beautiful needles, m.p. 114°. (Found: C, 59·66; H, 7·38; N, 4·08; OMe, 27·94. Mol wt 339 (Mass

spectrometry). $C_{14}H_{16}O_3N(OMe)_3$ requires: C, 60·18; H, 7·37; N, 4·10; OMe, 27·43% and mol wt 339.)

Acid hydrolysis of piperlongumic acid. A mixture of piperlongumic acid (0·5 g) and 10N H_2SO_4 (20 ml) was taken in a hard glass tube which was evacuated and sealed. The contents of the tube were heated in an oil bath (temp maintained at 120–125° for 6 hr). The seal was broken after cooling the tube and the aqueous acid soln extracted with ether (4 × 50 ml). The ether extract was washed with $NaHCO_3$aq. The aqueous bicarbonate extract was carefully acidified with dil HCl and then extracted with ether (4 × 50 ml). The ether extract was washed with water, dried and evaporated to dryness when a semisolid residue (0·2 g) was obtained. This was crystallized from dilute alcohol in fine needles, m.p. 125–126°. It recorded an undepressed mixed m.p. on admixture with an authentic sample of 3,4,5-trimethoxycinnamic acid.

Synthesis of α-piperidone. Cyclopentanoneoxime (5 g) was dissolved in 20 ml strong H_2SO_4 (100 ml conc acid and 20 ml water). It was then heated just to boiling when the solution became dark. The content was cooled and diluted with water (20 ml). The soln was then neutralized with 25% NaOHaq and extracted with chf (4 × 100 ml). The chf extract was washed with water, dried over Na_2SO_4 and the solvent removed. The resultant syrupy liquid was distilled under reduced press when α-piperidone (0·9 g) distilled at 105–110°/2–4 mm. On cooling it solidified into needles, m.p. 38–40°.

Acid hydrolysis of α-piperidone. α-piperidone (0·5 g) was refluxed with conc HCl (2 ml) for 2 hr. Excess acid was removed and the product (0·20 g) was crystallized from dry EtOH in fine needles, m.p. 84°. The compound was found to be identical with hydrochloride of 5-amino-n-valeric acid from its mixed m.p. with the authentic sample. (Found in a dry sample (dried over acetone): C, 43·68; H, 8·92; N, 10·21; Cl, 25·39. $C_5H_{11}ON{\cdot}HCl$ requires: C, 43·63; H, 8·72; N, 10·18; Cl, 25·81%.)

Condensation of 3,4,5-trimethoxycinnamoyl chloride with piperidine. An ethereal soln of 3,4,5-trimethoxycinnamoyl chloride (2 g) (prepared by the action of $SOCl_2$ and the acid) was added dropwise to a vigorously stirred soln of piperidine (1·9 g) in 10% NaOHaq (20 ml), kept at 0–5°. The stirring was continued for 1 hr more and left overnight. The product was extracted with ether (5 × 100 ml). The combined ether extract was washed successively with 2N HCl (4 × 25 ml), $NaHCO_3$aq (5%, 5 × 10 ml), water and dried. The solvent was distilled and the resultant brown semi-solid mass was crystallized from ether–pet. ether (b.p. 60–80°) (1:3) in fine colourless needles, m.p. 101–102°, yield, 1·20 g. (Found in a dry sample: C, 67·25; H, 7·57; N, 4·57; OMe, 30·58 $C_{17}H_{25}O_4N$ requires: C, 66·88; H, 7·54; N, 4·59; 3—OMe, 30·49%.)

3,4,5-*Trimethoxyphenylpropionamide of piperidine.* The condensation product (1 g) dissolved in abs EtOH (50 ml) was shaken in an H atm in presence of Adams catalyst (0·1 g), prereduced with H. Absorption of H (one mole) was complete after 2 hr. The catalyst was filtered off and the filtrate on removal of the solvent furnished a colourless oil, which solidified on cooling in ice, m.p. 70–75°. The product (0·6 g) was crystallized from acetone in beautiful colourless needles, m.p. 76–78°. (Found in a dry sample: C, 66·82; H, 8·18; N, 4·56; OMe, 30·86; M = 307 (Mass spectrometry). Calc. for $C_{17}H_{25}O_4N$: C, 66·45; H, 8·14; N, 4·58; 3-OMe, 30·29%; mol wt. 307.)

Tetrahydropiperlongumic acid (VI). To a suspension of piperidine amide of 3,4,5-trimethoxyphenylpropionic acid (0·5 g) in water (25 ml) $KMnO_4$aq (1 g in 20 ml water) was added dropwise with vigorous stirring at a temp of 60–70°. Stirring was continued for 4 hr. Excess $KMnO_4$ was decomposed by alcohol and filtered while hot. The residue was washed with hot water. The combined filtrate was concentrated (10 ml), cooled and acidified with dil HCl (pH 4–5) when an oily substance separated. The product was extracted with chf (4 × 50 ml), washed with water, dried and distilled. The resulting light brown oil was crystallized from AcOEt—ether (1:3) in shining needles, m.p. 114°; yield, 0·25 g. (Found in a dry sample: C, 59·68; H, 7·39; N, 4·06; OMe, 27·84.. Calc. for $C_{17}H_{25}O_6N$: C, 60·18; H, 7·37; N, 4·10; 3-OMe, 27·43%.)

Catalytic hydrogenation of piperlonguminine (VII). Piperlonguminine (0·137 g) dissolved in aldehyde-free EtOH (30 ml) was shaken for 3 hr in an H atm in presence of Adams catalyst (0·04 g), prereduced with H. H uptake during 3 hr corresponded to 2 mole equivs (25 ml) per mole of piperlonguminine and thus indicated the formation of a tetrahydroderivative. The alcoholic soln was filtered from the catalyst and the filtrate on removal of the solvent under reduced press furnished a colourless oil (0·11 g) which was crystallized from pet ether (b.p. 60–80°) in white needles, m.p. 67°.

(Found in a dry sample (dried over acetone): C, 69·25; H, 8·42; N, 5·10; M = 277 (Mass spectrometry). $C_{16}H_{23}O_3N$ requires: C, 69·31; H, 8·30; N, 5·05%; mol wt. 277.)

Ozonolysis of piperlonguminine. Piperlonguminine (0·3 g) was dissolved in AcOEt (40 ml) and AcOH (10 ml). The soln was cooled to 0° and a stream of ozonized oxygen (ca. 5% ozone) was bubbled through the soln till it contained excess O_3. A current of N was then passed through the soln at 0° until the outcoming gas no longer turned a starch iodide paper blue. The ozonide was then decomposed reductively by adding a mixture of Mg turnings (0·5 g) and aqueous AcOH (1:1, 10 ml) and the reaction mixture was kept overnight. The decomposition product was diluted with water (50 ml) and extracted with chf (4 × 50 ml).

The aqueous layer was removed and the organic layer was washed with 2N HCl to remove any basic material. The chf layer was washed with water, dried and distilled. The residue on distillation at 140°/15 mm yielded piperonal (0·1 g). It formed well defined DNPH derivative with alcoholic soln (5%) of 2,4-dinitrophenylhydrazine. The DNPH derivative was crystallized from EtOH in purplish-red crystals, m.p. 264–266°, undepressed when mixed with the authentic sample. (Found in a dry sample: C, 60·01; H, 3·08; N, 16·92. Calc. for $C_{14}H_{10}O_6N_4$: C, 50·90; H, 3·03; N, 16·97%.)

Acid hydrolysis of piperlonguminine. Piperlonguminine (0·2 g) was heated at 120° for 50 hr in a sealed tube with conc HCl (3 ml) and EtOH (5 ml). The seal was broken after cooling the tube and the acid soln after the removal of alcohol was extracted with ether (4 × 20 ml). The ether extract (B) and the acid extract (A) were separately treated.

Treatment of the acid extract (A) *and isolation of isobutylamine hydrochloride.* The aqueous acid solution (A) was evaporated to dryness and extracted with AcOEt (4 × 10 ml). The organic layer upon removal of the solvent furnished isobutylamine hydrochloride, (0·015 g). It crystallized from AcOEt in flakes, m.p. and mixed m.p. with an authentic sample, 172–173°. (Found in a dry sample: C, 43·62; H, 11·05; N, 12·92; Cl, 32·62. Calc. for $C_4H_{12}NCl$: C, 43·83; H, 10·96; N, 12·78; Cl, 32·42%.)

Treatment of the ether extract (B). The ether extract (B) was washed with water, dried and then evaporated to dryness when a brownish red solid (0·05 g) was obtained, m.p. above 340°. It could not be crystallized. The compound was found to be soluble in $NaHCO_3$aq and gave a catechol type colour reaction (light green) with alcoholic $FeCl_3$ soln.

Alkaline hydrolysis of piperlonguminine. Piperlonguminine (0·2 g) was dissolved in 40% alcoholic KOH (20 ml) by slight warming. The soln was heated under reflux for 8 hr. The solvent was removed under the reduced press and the viscous jelly thus obtained dissolved in water (20 ml) and extracted with ether (4 × 50 ml). The aqueous alkaline soln was acidified (congo red) with HCl. The flocculent ppt (0·1 g) (piperic acid) was filtered off, washed with water, dried and crystallized from MeOH in pale yellow needles, m.p. 217–218°. (Found: C, 65·97; H, 4·85; Calc. for $C_{12}H_{10}O_4$: C, 66·05; H, 4·81%.)

Piperic acid. Piperine (2·00 g), isolated from *Piper nigrum* Linn. was hydrolysed with 20% methanolic KOH (100 ml). The MeOH was removed under reduced press and the semisolid mass dissolved in water (50 ml). The aqueous soln upon acidification with 6N HCl yielded a flocculent yellowish ppt of piperic acid (1 g). The ppt was filtered off and crystallized from MeOH in yellow needles, m.p. 217–218° and found identical with a synthetic sample prepared from piperonal by the usual procedure.

Acid chloride of piperic acid. Piperic acid (1 g) was added portionwise to freshly distilled PCl_5 (9 ml) at 40–45° and the mixture heated at 60–70° for 3 hr. The supernatant liquid was decanted from the syrupy layer of phosphorus acid, when a brownish solid was obtained.

Condensation of piperic acid chloride with isobutylamine. Isobutylamine (1 g) was dissolved in dry benzene (50 ml) and pyridine (2 ml). A soln of piperic acid chloride (1 g) in dry benzene was added to the mixture slowly with stirring at a temp of 0–5°. The mixture was kept overnight. The reaction mixture was extracted thoroughly with benzene (4 × 50 ml). The benzene extract was washed successively with 2N HCl, then $NaHCO_3$aq and water. It was dried over Na_2SO_4 and concentrated to a brownish oil. The oil was chromatographed over Brockmann alumina using benzene and benzene–chf (3:1) as eluents. The benzene–chf eluates upon concentration furnished a white solid (0·6 g), m.p. 156–160°. The latter upon crystallization from benzene–petrol (2:1) furnished piperlonguminine. (Found: C, 69·53; H, 7·02; N, 5·20. calc. for $C_{16}H_{21}O_3N$: C, 70·33; H, 6·96; N, 5·12%.)

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