Chemical Examination of Tylophora asthmatica. *Part I.*

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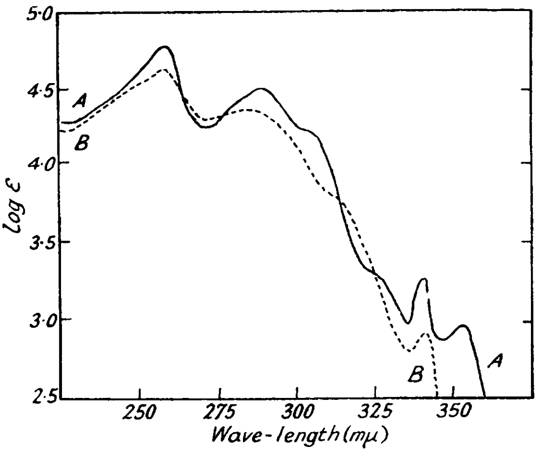
Two alkaloids, tylophorine and tylophorinine, have been isolated from *Tylophora asthmatica* and separated by chromatography. Tylophorine has been degraded by three successive Hofmann exhaustive methylations to a nitrogen-free product $C_{24}H_{24}O_4$. The flavone isolated from *Tylophora asthmatica* is shown to be kæmpferol.

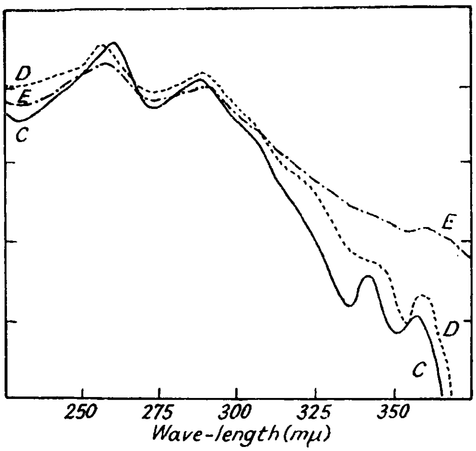
FROM *Tylophora asthmatica,* Wight et Arn., a perennial climber belonging to the natural order Asclepiadacæ, Ratnagiriswaran and Venkatachalam (*Indian J. Med. Res.*, 1935, **22,** 433) isolated two alkaloids, tylophorine and tylophorinine, to which they assigned the molecular formulæ, $C_{24}H_{27}O_4N$ and $C_{23}H_{27}O_4N$ respectively. Tylophorine was shown to have four and tylophorinine three methoxyl groups. Chopra, Ghosh, Bose, and Ghosh (*Arch. Pharm.*, 1937, **275,** 236) could isolate tylophorine from *T. asthmatica* but not tylophorinine, although they obtained a second, amorphous base, m. p. 125—130°, which was not further characterised. In the present study, both tylophorine and tylophorinine were isolated, in conformity with the finding of Ratnagiriswaran and Venkatachalam.

FIG. 1.



A, ———, Tylophorine. *B*, - - - -, Tylophorinine. (Both in ethanol.)

FIG. 2.



C, ———, Tylophorinemethine. *D*, - - - -, de-*N*-Methyltylophorinemethine. *E*, — · — · —, Nitrogen-free compound from tylophorine. (All in ethanol.)

A flavone, $C_{15}H_{10}O_6$, m. p. 268—269°, was also isolated and its identity with kæmpferol (Kostanecki, *Ber.*, 1901, **34**, 3723) was established.

Tylophorine and tylophorinine were separated by chromatography, without having recourse to the elaborate procedure of the earlier authors. Tylophorinine, when chromatographically purified, melts at 248—249° (decomp.), whereas the earlier authors report m. p. 232—233°. Further, tylophorinine nitrate has been reported to melt at 205—206° : the pure nitrate melts at 240—242°. The ultra-violet absorption spectra of tylophorine and tylophorinine are recorded in Fig. 1.

Tylophorine was found to contain no *N*-methyl group, but readily yielded a methiodide, $C_{24}H_{27}O_4N,CH_3I$, indicating the tertiary nature of the nitrogen atom. The methiodide was converted into a methohydroxide which at 100° *in vacuo* yielded tylophorinemethine, $C_{25}H_{29}O_4N$. A second Hofmann exhaustive methylation then yielded de-*N*-methyltylophorinemethine, $C_{26}H_{31}O_4N$, and a third application of this degradation gave a nitrogen-free product, $C_{24}H_{24}O_4$. The ultra-violet absorption spectra of these degradation products are recorded in Fig. 2. The evidence so far obtained indicates clearly that the nitrogen atom in tylophorine is common to two rings. The ready degradation to a nitrogen-free product by the Hofmann procedure also indicates the presence of three hydrogen atoms in β-positions with respect to the nitrogen atom. Further work is in progress.

EXPERIMENTAL

Extraction.—Powdered plant material (10 kg., whole plant) was extracted by percolation at room temperature with 90% alcohol containing 1% of acetic acid. The extract was concentrated to a syrup under reduced pressure. The dark brownish-green syrupy residue was extracted repeatedly with 0·5N-sulphuric acid until the acid extract no longer gave a precipitate with Mayer's reagent. The acid extract was concentrated to 1 l. *in vacuo*, filtered free from resin, and extracted repeatedly with ether. The ether and the aqueous layers were then separately worked up.

Isolation of the flavone. The ether extract, on removal of ether, yielded a residue containing acetic acid. On treatment with water (2 l.), a yellow crystalline material separated (1·2 g.). Crystallisation from dilute alcohol gave the flavone, m. p. 268—269° (decomp.), which gave a violet colour with neutral ferric chloride and a pink colour when treated with magnesium and acetic acid (Found : C, 62·4; H, 3·7. Calc. for $C_{15}H_{10}O_6$: C, 62·9; H, 3·5%), not depressed in m. p. on admixture with kæmpferol. The *tetra-acetyl* derivative, prepared by acetic anhydride and sodium acetate and crystallised from dilute alcohol, formed colourless needles, which softened at 120°, melted at 135°, resolidified above 135°, and melted again at 176° (Found : C, 58·8; H, 4·4. $C_{23}H_{18}O_{10},H_2O$ requires C, 58·5; H, 4·2%).

Isolation of the alkaloids. The acid extract was cooled, basified with ammonia, and extracted repeatedly with chloroform. The chloroform extract was dried (Na_2SO_4). On removal of the solvent a brownish solid (9 g.) was obtained. The crude alkaloidal mixture (1 g.) was dissolved in chloroform (30 ml.) and passed through a column of alumina (B.D.H. for chromatography) (100 g.). The column was developed with chloroform and the eluate was collected in nine 50-ml. portions. The column was then washed with ether–chloroform (1 : 1). Fractions 1—4 (respectively 0·28, 0·17, 0·09, and 0·05 g.) had m. p. 285—286°. Fraction 5 did not yield any material. Fractions 6—9 (each containing 0·01 g. of material) had m. p. 245°. Fraction 10 yielded material (0·02 g.) of m. p. 242°.

Tylophorine. Material obtained in fractions 1—4 separated from chloroform–alcohol as colourless crystals, m. p. 286—287° (decomp.), $[\alpha]_D^{27}$ $-11·6°$ (*c*, 1·07 in $CHCl_3$), $\lambda_{max.}$ 255, 290, 340, 352 mμ (log ε 4·74, 4·49, 3·30, 2·93) (Found : C, 73·0; H, 6·5; N, 4·0. Calc. for $C_{24}H_{27}O_4N$: C, 73·2; H, 6·9; N, 3·6%). The *nitrate*, prepared by digestion with hot 5% nitric acid in alcohol and recrystallised from water, melted at 265—267° (Found : C, 62·7; H, 6·1; N, 6·2. $C_{24}H_{28}O_7N_2$ requires C, 63·1; H, 6·2; N, 6·1%). The *methiodide*, prepared in quantitative yield by refluxing the base with excess of methyl iodide in chloroform solution for 1 hr. and crystallised from water, had m. p. 280° (Found : C, 55·6; H, 5·8; N, 2·5; I, 23·4. $C_{25}H_{30}O_4NI$ requires C, 56·0; H, 5·6; N, 2·6; I, 23·7%).

Tylophorinine. Material obtained from fractions 6—10 and crystallised from chloroform–alcohol had m. p. 248—249°, $[\alpha]_D^{27}$ $-14·2°$ (*c*, 1·76 in $CHCl_3$), $\lambda_{max.}$ 258, 287, 340 mμ (log ε 4·61, 4·34, 2·91) (Found : C, 72·0; H, 6·8. Calc. for $C_{23}H_{27}O_4N$: C, 72·4; H, 7·1%). The *nitrate* after crystallisation from water melted at 240—242° (Found: C, 61·8; H, 6·8. $C_{23}H_{28}O_7N_2$ requires C, 62·1; H, 6·3%).

Hofmann Degradation of Tylophorine.—(a) *Tylophorinemethine.* A suspension of tylophorine methiodide (2·5 g.) in water (300 ml.) was shaken with silver oxide (from 10 g. of silver nitrate) for 4 hr. The mixture was filtered and the filtrate evaporated at 45—50° *in vacuo* to dryness. The residue was heated at 100°/0·5 mm. for 30 min., and then extracted repeatedly with hot benzene. The filtered benzene extract after removal of solvent yielded the *methine* (1·45 g.), m. p. 185—186°. Recrystallisation from benzene yielded colourless needles, m. p. 190°, $\lambda_{max.}$ 261, 288, 343, 358 mμ (log ε 4·80, 4·53, 3·30, 3·10) (Found: C, 73·2; H, 7·3; N, 3·4. $C_{25}H_{29}O_4N$ requires C, 73·7; H, 7·1; N, 3·4%).

The *methiodide* was obtained by refluxing the methine (1·3 g.) in chloroform (5 ml.) with methyl iodide (5 ml.) for 4 hr. The precipitate (1·6 g.) was filtered off, washed with chloroform, and crystallised from methanol. It melted at 190° and decomposed above 250° (Found: C, 56·3; H, 6·0; N, 2·5; I, 23·6. $C_{26}H_{32}O_4NI$ requires C, 56·8; H, 5·8; N, 2·6; I, 23·1%).

(b) *de*-N-*Methyltylophorinemethine.* The methine methiodide (1·6 g.) was shaken in warm water (250 ml.) with silver oxide (from 10 g. of silver nitrate) for 5 hr. Further operations were conducted as previously. *De*-N-*methyltylophorinemethine* (1·02 g.), crystallised from 80% alcohol, sintered at 158° and melted at 167°, $\lambda_{max.}$ 258, 290, 344, 360 mμ (log ε 4·78, 4·58, 3·38, 3·18) (Found: C, 74·4; H, 7·3; N, 3·1. $C_{26}H_{31}O_4N$ requires C, 74·1; H, 7·4; N, 3·3%).

The *methiodide* was obtained by methyl iodide in chloroform at room temperature for a few hours. Crystallised from methanol–ether it melted at 196° (Found: C, 57·1; H, 6·0. $C_{27}H_{34}O_4NI$ requires C, 57·6; H, 6·0%).

(c) *Nitrogen-free product.* The foregoing methiodide (0·8 g.), when submitted to the Hofmann degradation as previously, yielded a nitrogen-free *product* (0·5 g.). Repeated crystallisations from benzene–light petroleum mixture yielded pale yellow crystals, m. p. 152—153°, $\lambda_{max.}$ 260, 290, 360 mμ (log ε 4·65, 4·50, 3·60) (Found: C, 75·9; H, 6·1. $C_{24}H_{24}O_4$ requires C, 76·6; H, 6·4%).

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