375. *An Alkaloid of* Dioscorea hispida, *Dennstedt. Part II.* Hofmann Degradation.*

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Further investigations relating to the constitution of the alkaloid from *Dioscorea hispida*, Dennst., are described. Hofmann degradation of the alkaloid has given an unsaturated, oxygen-free base, $C_{13}H_{21}N$, which has three double bonds, but is not aromatic. Additional chemical and pharmacological evidence supporting the view that the alkaloid is identical with dioscorine is recorded.

INVESTIGATIONS described in Part I * (see also *Nature*, 1951, **168**, 1090) have shown that the alkaloid occurring in the tubers of *Dioscorea hispida*, Dennst., contains an αβ-unsaturated six- or higher-membered lactone ring. In addition, chemical and botanical evidence was presented supporting the view, previously expressed by Leyva and Gutierrez (*J. Philippine Is. Med. Assoc.*, 1937, **17**, 349) on the basis of colour reactions and toxicological properties, that the alkaloid is identical with dioscorine, obtained by Schütte (*Nederl. Tijdschr. Pharm.*, 1897, **9**, 131) and Gorter (*Rec. Trav. chim.*, 1911, **30**, 161) from the tubers of *Dioscorea hirsuta*, Blume.

Additional chemical evidence supporting the belief that the base from *D. hispida* is dioscorine is now submitted. Hofmann degradation of the alkaloid from *D. hispida* gave two products, in approximately equal yield. The lower-boiling product was an oxygen-free base, characterised as the methiodide and picrate. Analyses supported the formula $C_{13}H_{21}N$ for the base, which is in agreement with the formula of the base obtained by Gorter (*loc. cit.*) by the Hofmann degradation of dioscorine. The properties of the two Hofmann bases are in agreement, except that Gorter's amine is reported as having an aniline-like odour, whereas the base obtained in this laboratory has an odour typical of an aliphatic or alicyclic amine.

The higher-boiling product was mainly the unchanged alkaloid, formed by elimination of methanol from the methohydroxide, but there was evidence that this fraction contained a small proportion of a more unsaturated compound, as in addition to the maximum in the ultra-violet absorption spectrum at 2170 Å, characteristic of the alkaloid (see Part I), there was a maximum at 2650 Å ($\varepsilon = 1700$), which suggests the presence of a diene system conjugated with a carbonyl group (Braude, *Ann. Reports*, 1945, **42**, 105). Such a grouping would almost certainly be found in the normal methine base of the alkaloid.

The base $C_{13}H_{21}N$ is evidently the result of deep-seated decomposition of the methohydroxide of the alkaloid, and the presence of the nitrogen atom indicates that in the alkaloid this atom is part of a heterocyclic ring. The elimination of the lactone ring recalls Boekelheide and Agnello's recent finding (*J. Amer. Chem. Soc.*, 1951, **73**, 2286) that the lactonic alkaloid β-erythroidine, when converted into its dihydro-derivative and subjected to Hofmann degradation, furnished an oxygen-free base as well as the normal methine base. The base $C_{13}H_{21}N$ is formed by the loss of water and carbon dioxide from the methohydroxide; if it be assumed that one double bond is formed for the elimination of each of these simple molecules, the base may be expected to contain three ethylenic linkages. That this was the case was proved by catalytic hydrogenation of the base under mild conditions, using a pre-reduced catalyst. Three mols. of hydrogen were absorbed, with the formation of a saturated hexahydro-base $C_{13}H_{27}N$, characterised as the methiodide. It may be concluded that the three double bonds are not present as a benzene ring; in confirmation ozonolysis of the base gave formaldehyde in high yield, indicating the presence of a terminal methylene group. The infra-red absorption spectrum of the base (bands at 3·23, 5·80, and 11·26 μ) is consistent with this. In addition, bands at 5·61 and 5·73 μ, and the absence of strong bands between 10 and 11 μ, suggest that this grouping is of the type $\begin{matrix}C\\C\end{matrix}\!>\!C\!:\!CH_2$ rather than $CH\!:\!CH_2$ (Thompson, *J.*, 1948, 328; Williams, *Rev. Sci. Instr.*, 1948, **19**, 143; cf. Boekelheide, Grundon, and Weinstock, *J. Amer. Chem. Soc.*,

* Part I, *J.*, 1952, 2236.

1952, **74**, 1866). All these bands are absent from the spectrum of the hexahydro-base $C_{13}H_{27}N$.

The ultra-violet absorption curve of the base $C_{13}H_{21}N$ shows a single wide maximum at 2700 Å ($\varepsilon = 17{,}500$), which suggests that the three double bonds of the base are conjugated. Cases for comparison include *cyclo*octa-1 : 3 : 5-triene [max. at 2650 Å ($\varepsilon = 3715$) (Cope and Overberger, *J. Amer. Chem. Soc.*, 1948, **70**, 1433; Cope, Nace, and Estes, *ibid.*, 1950, **72**, 1123)], octa-2 : 4 : 6-triene [max. at 2600 Å ($\varepsilon = 7940$) (Morton, "The Application of Absorption Spectra to the Study of Vitamins, Hormones, and Coenzymes," Adam Hilger, Ltd., London, 2nd Edn., 1942, p. 25)], and octa-2 : 4 : 6-trien-1-ol [max. at 2645 Å ($\varepsilon = 53{,}000$) (von Euler, Karrer, Klussman, and Morf, *Helv. Chim. Acta*, 1932, **15**, 502)]. The width of the maximum and the low melting point (120—122°) of the methiodide of the base suggest that the base is probably a mixture of conjugated trienes, arising because of the unsymmetrical placing of the nitrogen atom, especially with respect to the lactone ring, so that the elements of water may be eliminated from the methohydroxide in different ways. It is unlikely that in any of these structures for the base $C_{13}H_{21}N$ the nitrogen atom is attached to the chromophoric system since a nitrogen atom so placed is associated with a large bathochromic effect (*e.g.*, buta-1 : 3-diene, $\lambda_{max.}$ 2190 Å; 1-diethylaminobuta-1 : 3-diene, $\lambda_{max.}$ 2810 Å; Braude, *loc. cit.*).

The hexahydro-base $C_{13}H_{27}N$ forms a methiodide, m. p. 182—183°, but it has not so far been possible to establish whether this base is homogeneous. The infra-red absorption spectrum of the base shows a wide band of medium intensity at 11·75—12·1 μ, which suggests the presence of a trimethylene group (Thompson, *loc. cit.*).

Investigations of the structure of the two bases are being continued. If it can be assumed that the base $C_{13}H_{21}N$ is identical with that obtained by Gorter, it seems likely that it is a mixture of alkenyldimethylamino*cyclo*heptadienes, since Gorter (*loc. cit.*) reported the conversion of the base, by further Hofmann degradation, hydrobromination, dehydrobromination, and oxidation, into *o*-toluic acid.

Reduction of the dihydro-alkaloid with lithium aluminium hydride gave a syrupy glycol-base, with a strong band in the infra-red at 3·0 μ, characteristic of the hydroxyl group.

Extraction of tubers of *D. hispida* according to the procedure of Marker and his co-workers (*J. Amer. Chem. Soc.*, 1940, **62**, 2542; 1942, **64**, 1283) failed to yield sapogenins. It has also been established that the tubers of *Dioscorea macrostachia* and of *D. composita* (kindly supplied by Professor Carl Djerassi, late of Syntex, S.A., Mexico) do not contain any alkaloidal material.

Pharmacology.—The author is grateful to Mr. A. F. Green of the Wellcome Research Laboratories for the following pharmacological report.

Analeptic and convulsant actions were shown when the alkaloid was given in large doses. The duration of anæsthesia of mice under pentobarbitone sodium was shortened by 50 mg./kg. given intravenously, but less than by 1—2 mg./kg. of picrotoxin. In anæsthetised mice convulsions occurred with 50 mg./kg., and 100 mg./kg. were usually fatal. It was also found that 20 mg./kg. intravenously increased the breathing and lightened the degree of anæsthesia in a cat under pentobarbitone sodium. No other actions of importance were found. Intraperitoneally in mice the LD_{50} was about 120 mg./kg. There was no analgesic effect with large doses in rats. Concentrations of $1 : 10^{-5}$ did not affect the movement of the isolated guinea-pig or rabbit ileum, or their responses to acetylcholine and histamine. The cat blood pressure was hardly affected by 20 mg./kg. intravenously, and its responses to acetylcholine, histamine, adrenaline, and noradrenaline were not influenced. Mydriatic activity could not be demonstrated either by direct application of a 0·1% solution to the eyes of mice or by intraperitoneal injection of as much as 50 mg./kg. Atropine under these conditions is active in 0·0005% solution or at 0·05 mg./kg. The alkaloid caused mydriasis only at toxic doses. The dihydro-alkaloid showed no pharmacological effects.

The properties of the alkaloid described above are in general similar to those of dioscorine, which have been reported earlier somewhat scantily. Thus Schütte (*loc. cit.*) reported that dioscorine had picrotoxin-like properties and Gorter (*loc. cit.*) confirmed this,

noting that large doses were required. Leyva and Gutierrez (*loc. cit.*) noted similar toxicological effects with the alkaloid of *D. hispida*, but unfortunately their finding that a monkey dying after a large dose of the alkaloid had dilated pupils has been misused as evidence for a mydriatic effect, resembling that of tropine, when it might have been better regarded as due to asphyxia or some other agonal effect. There are therefore no pharmacological data to indicate that the alkaloid of *D. hispida* investigated in this laboratory is different from dioscorine itself.

Experimental

Most of the analyses are by Mr. F. C. Hall. Spectral measurements are by Dr. F. B. Strauss, with the assistance of Mr. F. Hastings.

Hofman Degradation of the Alkaloid.—The crystalline alkaloid (2·0 g.) in dry methanol (10 c.c.) and methyl iodide (5 c.c.) were refluxed on the water-bath for 3 hr. After removal of the solvent and excess of methyl iodide *in vacuo* the residual crystalline methiodide (3·3 g., 100%) was dissolved in water (15 c.c.) and the solution treated with freshly precipitated silver oxide, prepared from silver nitrate (3·0 g.) and N-sodium hydroxide (50 c.c.). After 12 hr. with occasional shaking the solution was filtered and the filtrate evaporated to dryness *in vacuo* on the water-bath. The foamy methohydroxide (2·1 g.) so obtained decomposed smoothly at 180—220°/12 mm., giving an aqueous distillate (1·4 g.), which was heated under reflux on the water-bath with 20% aqueous potassium hydroxide (20 c.c.) for 1 hr. The cooled solution was extracted several times with ether, and the combined extracts were dried (KOH). Removal of the ether through a short Vigreux column gave an oily base, which distilled at 125° (bath)/8 mm. (0·6 g.) and had $[\alpha]_D^{18}$ −185° (*c*, 1·0 in $CHCl_3$) (Found: C, 81·9; H, 11·25; N, 7·55; CMe, 6·0. Calc. for $C_{13}H_{21}N$: C, 81·7; H, 11·0; N, 7·3; 1*C*-Me, 7·85%). Gorter (*loc. cit.*) reports b. p. of Hofmann base from dioscorine 116—120°/8 mm. Ultra-violet absorption in ethanol: Max. at 2700 Å, ε = 17,500. Infra-red absorption (liquid film): Bands at 3·23, 5·61, 5·73, 5·80, 6·11, and 11·26 μ.

A *methiodide* was obtained by refluxing the Hofmann base (0·1 g.) with methyl iodide (2 c.c.) for 10 min.; it separated from ethanol–ether in clusters of glistening prisms, m. p. 120—122° (Found: C, 50·2; H, 7·0; I, 38·0. $C_{14}H_{24}NI$ requires C, 50·45; H, 7·2; I, 38·1%). A *picrate*, prepared in the usual manner in methanol, separated from methanol in rhombic prisms, m. p. 129—130° (Found: C, 54·5; H, 5·6. $C_{19}H_{24}O_7N_4$ requires C, 54·3; H, 5·7%).

Acidification of the potassium hydroxide solution above, followed by basification with sodium carbonate and extraction with chloroform, gave a base distilling at 140—150° (bath)/0·06 mm. (0·7 g.), which was mainly unchanged alkaloid (picrate, m. p. and mixed m. p. 187°), but the appearance in the ultra-violet absorption spectrum of a peak at 2650 Å (ε = 1,700) indicates that the product contains some of the normal methine base of the alkaloid.

Ozonolysis of the Base $C_{13}H_{21}N$.—A solution of the base $C_{13}H_{21}N$ (0·25 g.) in purified chloroform (20 c.c.) was ozonised at room temperature for 3 hr. Evaporation of the solvent *in vacuo* at room temperature gave an oily ozonide, which was decomposed by distillation with normal hydrochloric acid (50 c.c.), about 40 c.c. of distillate being collected. The distillate, with an excess of a saturated solution of 2 : 4-dinitrophenylhydrazine in 2N-hydrochloric acid, gave formaldehyde 2 : 4-dinitrophenylhydrazone, yellow needles (from ethanol) (0·20 g.), m. p. and mixed m. p. 160—161°.

Catalytic Hydrogenation of the Base $C_{13}H_{21}N$.—Adams' platinum oxide catalyst (*ca.* 50 mg.) was suspended in N-hydrochloric acid (10 c.c.) and pre-reduced by shaking in hydrogen at room temperature and pressure for 30 min. A solution of the base $C_{13}H_{21}N$ (0·20 g.) in N-hydrochloric acid (10 c.c.) was then added and the shaking resumed. After 1 hr. absorption had ceased (at 17°/760 mm., 78 c.c.; calc. for $3H_2$, 77 c.c.). The solution was filtered and basified with potassium hydroxide, and the product isolated with ether. The dried ethereal solution was evaporated through a short Vigreux column, and the residual oily *base* distilled, b. p. 125—128° (bath)/12 mm., 110° (bath)/9—10 mm. (0·20 g.), $[\alpha]_D^{19}$ −36·7° (*c*, 1·5 in $CHCl_3$) (Found: C, 79·1; H, 13·9; N, 7·1; CMe, 6·55, 7·05. $C_{13}H_{27}N$ requires C, 79·2; H, 13·7; N, 7·1; 1*C*-Me, 7·6%). The base did not decolorise dilute, aqueous permanganate at room temperature in 30 sec. Infra-red absorption (liquid film): Band of medium intensity at 11·75—12·1 μ.

A *methiodide* was obtained by refluxing the base (0·1 g.) with methyl iodide (2 c.c.) for 10 min.; evaporation of volatile matter furnished a syrup, which crystallised on trituration with acetone. The salt separated from the same solvent in clusters of elongated prisms, m. p. 182—183° (Found, on material dried at 100° *in vacuo*: C, 49·9; H, 8·8; I, 37·4. $C_{14}H_{30}NI$ requires C, 49·6; H, 8·8; I, 37·5%).

Lithium Aluminium Hydride Reduction of the Dihydro-alkaloid.—The dihydro-alkaloid, obtained as described previously, had $[\alpha]_D^{20}$ 0° (*c*, 3·1 in $CHCl_3$) and its syrupy *hydrochloride* had $[\alpha]_D^{26}$ +20° (*c*, 1·8 in H_2O). The dihydro-base (1·0 g.) in dry ether (50 c.c.) was slowly added with shaking to a suspension of powdered lithium aluminium hydride (0·7 g.) in dry ether (75 c.c.) during 20 min. After 3 hr. the mixture was refluxed on the water-bath for a further 2 hr., then decomposed with moist ether with ice-cooling and with " Celite 545 " as coagulant. Water (20 c.c.) was added, followed by 30% aqueous potassium hydroxide (100 c.c.). The mixture was then subjected to continuous ether-extraction for 16 hr. The dried extract was evaporated and the syrupy basic *glycol* distilled [b. p. 155—160° (bath)/0·01 mm.; 0·9 g.] (Found : C, 69·0; H, 11·1. $C_{13}H_{25}O_2N$ requires C, 68·7; H, 11·0%). The product was a colourless, viscous liquid, very soluble in water. Infra-red absorption (liquid film) : Strong band at 3·0 μ.

A *picrate*, prepared in the usual manner in methanol, separated from the same solvent in clusters of yellow prisms, m. p. 140—144°, with previous softening at 137° (Found, on material dried at 80° *in vacuo* : C, 50·3; H, 6·1. $C_{19}H_{28}O_9N_4$ requires C, 50·0; H, 6·1%).

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