

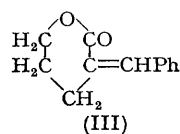
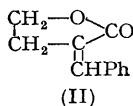
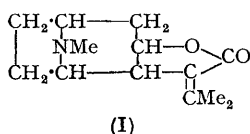
**418.** *An Alkaloid of Dioscorea hispida, Dennstedt. Part I.  
The Lactone Ring.*

By A. R. PINDER.

Investigations are described bearing on the constitution of the alkaloid from *Dioscorea hispida*, Dennst. Chemical evidence suggests that the alkaloid is identical with dioscorine. Degradative and spectroscopic experiments indicate that the alkaloid has a six- or higher-membered  $\alpha\beta$ -unsaturated lactone ring. A preliminary account of this investigation has appeared elsewhere (Pinder, *Nature*, 1951, **168**, 1090).

THE alkaloid dioscorine was first isolated by Boorsma (*Meded. uit's Lands Plant.*, 1894, XIII) from the tubers of *Dioscorea hirsuta*, Blume, known in Java as "gadoeng." Later, the base was obtained crystalline by Schütte (*Nederl. Tijdschr. Pharm.*, 1897, **9**, 131; *Chem. Zentr.*, 1897, II, 130), who also prepared several crystalline salts. In 1911, Gorter

(*Ann. Jard. Bot. Buit.*, Series 2, Supplement 3, p. 385; *Rec. Trav. chim.*, 1911, **30**, 161) carried out degradative investigations on the alkaloid, and suggested that it was a derivative of tropane, with structure (I).



Some years later, Leyva and Gutierrez (*J. Philippine Is. Med. Assoc.*, 1937, **17**, 349) isolated from tubers of *Dioscorea hispida*, Dennst., known in the Philippine Islands as "nami," an alkaloid having the same toxicological properties and colour reactions as dioscorine. They considered their product to be identical with dioscorine, though the base was not obtained crystalline, nor were any derivatives described.

In this laboratory, the extraction of the alkaloid from *D. hispida* has been repeated by a modified procedure. A comparison of the properties and reactions of this base with those reported for dioscorine (Schütte, *loc. cit.*; Gorter, *loc. cit.*) shows that in all probability the two substances are identical, although there are, however, some slight discrepancies between the melting points (see Table). It is possible that the earlier workers did not obtain the alkaloid in a pure condition.

There is in the botanical literature some confusion in the nomenclature of these two *Dioscoreae*. It is generally considered that *D. hirsuta*, Blume and *D. hispida*, Dennst. are synonymous, although five varieties of *D. hispida* are accepted, and it is possible that they may differ biochemically [Burkill, *Garden's Bull. Straits Settlements*, 1924, **3**, (4-6), pp. 121-244; Prain and Burkill, *Ann. Roy. Bot. Garden Calcutta*, 1937]. Unfortunately it has not so far proved possible to obtain authentic specimens of either dioscorine or *D. hirsuta*, Blume, so that a direct comparison has not been made.

The base from *D. hispida*, purified by distillation *in vacuo*, crystallised readily and formed beautifully crystalline salts, all of which gave analytical figures in support of a molecular formula  $\text{C}_{13}\text{H}_{19}\text{O}_2\text{N}$  for the base. The base is very soluble in water, to give a strongly alkaline solution; with ammoniacal silver nitrate no reduction occurs in five minutes, and after 48 hours only a faint precipitate. The presence of a lactone ring is clearly demon-

	Dioscorine, m. p.	Alkaloid from <i>D. hispida</i> , m. p. (uncorr.)
Base .....	43.5° (i)	54-55°
Hydrochloride .....	204 (i)	210-211 (decomp.)
Hydrobromide .....	213-214 (ii)	207
Methiodide .....	213 (ii)	207 (decomp.)
Picrate .....	183-184 (i)	187 (decomp.)

(i) Schütte, *loc. cit.*

(ii) Gorter, *loc. cit.*

strated by the behaviour of the base towards alkali (see Experimental section). Two colour reactions described for dioscorine, *viz.*, with alkaline sodium nitroprusside and with potassium iodate and sulphuric acid, are shown by the base.

A Herzig-Meyer determination reveals the presence of one NMe group, and the ease with which the base forms a methiodide with one molecular proportion of methyl iodide suggests that it is tertiary. A Kuhn-Röth oxidation shows the presence of one CMe.

The base reacts immediately with cold potassium permanganate solution. Oxidation of a hot, acid solution of the base by Reynolds and Robinson's procedure (*J.*, 1934, 594) gives formaldehyde only, in good yield. From this it may be inferred that either the base contains a side-chain methylene ( $\text{=CH}_2$ ) group, or, more probably, that the NMe group is oxidised, providing formaldehyde and the demethyl compound, as in the permanganate oxidation of tropine to nortropine (Merling, *Annalen*, 1883, **216**, 340; Willstätter, *Ber.*, 1896, **29**, 1579). The latter explanation was verified by ozonolysis: treatment of an acetic acid solution of the alkaloid with ozone for two hours gave no volatile product, but after six hours a small yield of formaldehyde was obtained, again as the sole product, in all probability by formation and decomposition of the amine oxide (cf. Polonovski and

Polonovski, *Bull. Soc. chim.*, 1927, **41**, 1190; Bailey and Robinson, *J.*, 1948, 704). That the presence of an unsaturated linkage in a side-chain would have been shown by the ready formation of a volatile aldehyde or ketone on ozonolysis was confirmed by the behaviour of  $\alpha$ -benzylidene- $\gamma$ -butyrolactone (II) and  $\alpha$ -benzylidene- $\delta$ -valerolactone (III): both compounds gave benzaldehyde readily on oxidation or ozonolysis. Attempts to prepare the corresponding isopropylidene compounds, as more suitable models, have so far failed.

Hydrogenation of the alkaloid with platinum oxide in ethanol gave a liquid dihydro-base, stable to cold aqueous permanganate for several minutes; its behaviour towards alkali indicated that a lactone ring was still present. It formed a series of beautifully crystalline salts, analysis of which supported the molecular formula  $C_{13}H_{21}O_2N$  for the dihydro-base.

The ultra-violet absorption curve for the alkaloid shows a single maximum at 2170 Å ( $\epsilon_{\text{molar}} = 16,160$ ), suggesting the presence of an ethylenic bond conjugated with the carbonyl group of a carboxylic acid, ester, or lactone (cf. Braude, *Ann. Reports*, 1945, **42**, 113). The curve for  $\delta$ -hexenolactone is very similar, with a maximum at 2140 Å ( $\epsilon_{\text{molar}} = 6700$ ). The dihydro-alkaloid shows no absorption at wave-lengths greater than 2140 Å, which is consistent with the saturation of the double bond during catalytic reduction of the alkaloid;  $\delta$ -hexanolactone also shows no absorption in this region.

The infra-red absorption spectra of the alkaloid, the dihydro-alkaloid, and several model substances have been measured (solids as a paste with "Nujol"; liquids as films). The results are summarised in the Table. The relatively low-frequency band of 1712  $\text{cm}^{-1}$

	Carbonyl stretching vibration frequency ( $\text{cm}^{-1}$ )
Alkaloid .....	1712
Dihydro-alkaloid .....	1730
$\alpha$ -Benzylidene- $\gamma$ -butyrolactone (II) .....	1742
$\alpha$ -Benzylidene- $\delta$ -valerolactone (III) .....	1712
$\delta$ -Hexenolactone .....	1725
$\delta$ -Hexanolactone .....	1733
$\gamma$ -Butyrolactone .....	1770

shown by the alkaloid, compared with the value of 1742  $\text{cm}^{-1}$  for  $\alpha$ -benzylidene- $\gamma$ -butyrolactone and of 1750  $\text{cm}^{-1}$  for an  $\alpha\beta$ -unsaturated five-membered lactone ring (Grove and Willis, *J.*, 1951, 877), suggests that the lactone ring in the alkaloid is not five-membered, but six- or higher-membered. This view is strengthened by the existence of a band at 1712  $\text{cm}^{-1}$  in the spectrum of  $\alpha$ -benzylidene- $\delta$ -valerolactone, although chemical evidence rules out the possibility of there being an exocyclic double bond in the alkaloid. The infra-red spectra of  $\alpha\beta$ -unsaturated lactones have not been extensively investigated, so models for comparison are few.  $\delta$ -Hexenolactone has a band at 1725  $\text{cm}^{-1}$ , rather higher than for the alkaloid; this may be due to the alkaloid's containing a seven-membered lactone ring. The dihydro-alkaloid has a band at 1730  $\text{cm}^{-1}$ , almost the same as the band for  $\delta$ -hexanolactone (1733  $\text{cm}^{-1}$ ). These values are slightly different from the frequency of 1740  $\text{cm}^{-1}$ , characteristic of six- or higher-membered saturated lactones or saturated esters (Grove and Willis, *loc. cit.*; Rasmussen and Brattain, *J. Amer. Chem. Soc.*, 1949, **71**, 1073); the difference may perhaps be accounted for by the presence of substituents in the lactone ring. Whatever the explanation, the band for the dihydro-base is nearer to that of  $\delta$ -hexanolactone than to that of  $\gamma$ -butyrolactone (1770  $\text{cm}^{-1}$ ), which suggests that the lactone ring in the alkaloid is six- or higher-membered. Owing to the absence of strain in six- or higher-membered saturated lactone rings, it is not possible to distinguish between, say, a six- and a seven-membered ring by a comparison of carbonyl stretching frequencies.

In addition to the carbonyl band, the alkaloid shows a small band at 851  $\text{cm}^{-1}$ , which is absent from the spectrum of the dihydro-alkaloid. This suggests the presence in the alkaloid of the grouping  $\text{CRR}'\text{:CHR}''$  (Thompson and Torkington, *Proc. Roy. Soc.*, 1945, *A*, **184**, 3; Thompson, *J.*, 1948, 328), so that a system  $\text{CHR}'\text{:CR}''\text{:CO}\cdot\text{O}\cdot\text{C}\leq$  or  $\text{CRR}'\text{:CH}\cdot\text{CO}\cdot\text{O}\cdot\text{C}\leq$  is present.

The investigations are being continued.

## EXPERIMENTAL

M. p.s and b. p.s are uncorrected.

*Extraction of the Alkaloid.*—Fresh tubers of *D. hispida*, Dennst. (300 g.), were freed from roots and pulped in the presence of ethanol (285 c.c.) and acetic acid (15 c.c.). After being kept three days with occasional stirring, the combined mash from ten such operations was filtered, the residue being washed thoroughly with ethanol. The combined filtrate and washings were evaporated on the water-bath under reduced pressure, foaming being countered by frequent additions of *n*-octyl alcohol. The residual dark-brown syrup was taken up in water (400 c.c.), and the solution filtered and basified with an excess of sodium carbonate. The clear, brown solution was extracted with chloroform (6 × 100 c.c.), a separation being effected with the aid of a centrifuge. The combined extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated, the residual dark syrup being distilled *in vacuo*. The alkaloid was obtained as a pale yellow syrup, distilling at 135—150° (bath)/0.04 mm. It was purified by two further distillations at 140—145° (bath)/0.04 mm. (2—3 g.) and then gradually crystallised. It separated from a small volume of ether in colourless, elongated prisms, m. p. 54—55°,  $[\alpha]_D^{25} = -35.0^\circ$  (*c*, 3.4 in chloroform) (Found: C, 70.6, 70.6; H, 8.5, 8.6; N, 6.2, 6.3; CMe, 6.2; NMe, 6.5. Calc. for C<sub>13</sub>H<sub>19</sub>O<sub>2</sub>N: C, 70.6; H, 8.6; N, 6.3; 1CMe, 6.8; 1NMe, 6.8%) (cf. Gorter, *loc. cit.*; Leyva and Gutierrez, *loc. cit.*).

The alkaloid gave a reddish-violet colour with alkaline sodium nitroprusside solution, and a violet colour with potassium iodate and sulphuric acid. When an aqueous solution of the alkaloid was basified in the cold with excess of potassium hydroxide, and extracted with chloroform, the alkaloid was recovered. If, however, the basified solution was heated for an hour on the water-bath, or kept for 24 hours, chloroform extraction gave no alkaloid. Further, if the basified solution was acidified and heated for a while, then cooled and re-basified with an excess of sodium carbonate and extracted with chloroform, the alkaloid was regenerated.

The hydrochloride separated from alcohol-ether in nodules, m. p. 210—211° (decomp.) (Found: C, 60.5; H, 7.9; Cl, 13.7. Calc. for C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>NCl: C, 60.6; H, 7.8; Cl, 13.8%). The hydrobromide crystallised from a large volume of acetone in small, hard prisms, m. p. 207° (Found: C, 51.6; H, 6.6; Br, 26.4. Calc. for C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>NBr: C, 51.65; H, 6.6; Br, 26.5%). The picrate crystallised from acetone in glistening, yellow prisms, m. p. 187° (decomp.) (Found: C, 50.9; H, 4.9; N, 12.5. Calc. for C<sub>19</sub>H<sub>22</sub>O<sub>8</sub>N<sub>4</sub>: C, 50.7; H, 4.9; N, 12.4%). The methiodide separated from ethanol in hard prisms, m. p. 207° (decomp.) (Found: C, 46.4; H, 6.4; I, 34.8. Calc. for C<sub>14</sub>H<sub>22</sub>O<sub>2</sub>NI: C, 46.3; H, 6.1; I, 35.0%).

*Permanganate Oxidation of the Alkaloid.*—A solution of the alkaloid (0.1 g.) in 5% sulphuric acid (50 c.c.) was gently boiled in a flask fitted with a dropping funnel and an efficient downward condenser. Potassium permanganate solution (80 c.c. of 1.5%) was added dropwise to the boiling solution during 5 minutes, with simultaneous gentle distillation. A further quantity (80 c.c.) of the permanganate solution was added as before, a total of about 90 c.c. of distillate being collected. The distillate, in 2*N*-sulphuric acid, gave, during several hours, formaldehyde 2 : 4-dinitrophenylhydrazone as orange-yellow needles (from ethanol), m. p. and mixed m. p. 163°. M. p. 135—140° was observed on admixture with acetaldehyde 2 : 4-dinitrophenylhydrazone (m. p. 162—163°) and 110—115° with acetone 2 : 4-dinitrophenylhydrazone (m. p. 126°).

*Ozonolysis.*—A solution of the alkaloid (1.0 g.) in glacial acetic acid (15 c.c.) was ozonised for 2 hours at 15°, the exhaust gases being passed through a series of cooled water-traps. The solution was diluted with water (100 c.c.) and distilled, about 70—80 c.c. being collected. Treatment of the distillate with 2 : 4-dinitrophenylhydrazine solution gave a faint turbidity, but no precipitate. The aqueous solutions from the traps also gave no precipitate when treated with the reagent. Basification of the cooled, residual acetic acid solution with sodium carbonate, followed by chloroform extraction, gave unchanged alkaloid (0.9 g.) which, purified by distillation and crystallisation, had m. p. and mixed m. p. 54°.

When the ozonolysis was conducted for 6 hours under these experimental conditions, a small yield of formaldehyde 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 163°, was obtained.

*Hydrogenation.*—A solution of the alkaloid (0.5 g.) in ethanol (10 c.c.) was shaken with Adams's catalyst in hydrogen at room temperature and pressure. After 6½ hours, absorption ceased (1 mol.). The solution was filtered and evaporated, and the residual oil distilled *in vacuo*. The *dihydro*-compound distilled at 135—140° (bath)/0.06 mm. (0.5 g.) (Found: C, 69.8; H, 9.55; N, 6.2. C<sub>13</sub>H<sub>21</sub>O<sub>2</sub>N requires C, 70.0; H, 9.4; N, 6.3%). It was a clear, colour-

less syrup, very soluble in water to a strongly alkaline solution. It behaved towards alkalis as did the parent alkaloid, indicating that a lactone ring was still present. The *picrate* separated from ethanol in yellow prisms, m. p. 164—165° (Found: C, 50.3; H, 5.5.  $C_{19}H_{24}O_9N_4$  requires C, 50.4; H, 5.3%). The *methiodide* crystallised from ethanol in clusters of hard prisms, m. p. 229—230° with previous darkening (Found: C, 45.8; H, 6.5; I, 34.6.  $C_{14}H_{24}O_2NI$  requires C, 46.0; H, 6.6; I, 34.8%).

*α-Benzylidene-γ-butyrolactone* (II).—To a suspension of alcohol-free sodium ethoxide (from 2.3 g. of sodium) in absolute ether (50 c.c.) was added *γ*-butyrolactone (8.6 g.) in ether (20 c.c.), with shaking, followed by a solution of benzaldehyde (10.6 g.) in ether (20 c.c.). The mixture was kept for 1 hour with occasional shaking. Water (12.5 c.c.) was added, followed by dilute sulphuric acid (50 c.c.). The organic layer was separated, washed with saturated sodium hydrogen sulphite solution and water, dried, and evaporated. Distillation of the ether gave *α-benzylidene-γ-butyrolactone* (3.0 g.), which distilled at 155—160° (bath)/0.1 mm., crystallised readily, and separated from light petroleum (b. p. 60—80°) in pale cream elongated prisms, m. p. 116—117° (Found: C, 75.8; H, 6.0.  $C_{11}H_{10}O_2$  requires C, 75.9; H, 5.75%) (cf. Losanitsch, *Sitzungb. Akad. Wiss. Wien*, 1914, **123**, 13). Oxidation of the lactone with acid permanganate or ozonolysis gave benzaldehyde in high yield. The lactone had  $\lambda_{max}$ . 2800 Å ( $\epsilon_{molar}$  = 28,200).

*α-Benzylidene-δ-valerolactone* (III).—A similar experiment with *δ*-valerolactone (5.0 g.; prepared by the perbenzoic acid oxidation of cyclopentanone, Friess, *J. Amer. Chem. Soc.*, 1949, **71**, 1571), sodium ethoxide (from 1.2 g. of sodium), and benzaldehyde (5.3 g.) gave *α-benzylidene-δ-valerolactone* (3.5 g.), b. p. 150—155° (bath)/0.02 mm., which separated from light petroleum (b. p. 60—80°) in glistening plates, m. p. 58° (Found: C, 76.3; H, 6.4.  $C_{12}H_{12}O_2$  requires C, 76.6; H, 6.4%), and had  $\lambda_{max}$ . 2800 Å ( $\epsilon_{molar}$  13,800). Oxidation with acid permanganate or ozonolysis gave benzaldehyde in high yield.

*δ-Hexenolactone*.—This unsaturated lactone, b. p. 98°/10 mm., was prepared from sorbic acid as described by Kuhn and Jerchel (*Ber.*, 1943, **76**, 413). It did not reduce ammoniacal silver nitrate in 5 minutes (cf. Kuehl, Linstead, and Orkin, *J.*, 1950, 2214), and was oxidised immediately in the cold by aqueous permanganate.

*δ-Hexanolactone*.—Catalytic hydrogenation of *δ*-hexenolactone gave *δ*-hexanolactone (Kuhn and Jerchel, *loc. cit.*), b. p. 100—101°/10 mm., stable to cold, aqueous permanganate.

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