407. *The Alkaloids of* Virgilia oroboides.

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The seeds of *Virgilia oroboides* (Berg.) Salter (Leguminosae) have been found to contain five alkaloids, one of which, virgiline (I), to which the formula $C_{16}H_{26}N_2O_2$ had previously been ascribed, has now been shown to have the formula $C_{15}H_{24}N_2O_2$ and is in fact (−)-13-hydroxyaphylline. A second base, calpurnine (II), is an ester of pyrrole-2-carboxylic acid. Of the remaining bases, one occurs in small amount and has been shown to be epilupinine, and the other two, which occur only as traces, appeared from filter-disc paper chromatography to be 13-hydroxylupanine and cytisine.

It has been reported by White [1] that the tops of *Virgilia capensis* Lam. contain at least three alkaloids. Of these a new base, virgiline, was given the formula $C_{16}H_{26}N_2O_2$ and was shown to contain one basic nitrogen atom, an inert carbonyl group, and a secondary hydroxyl group. White suggested that virgiline was probably allied to lupanine, having in addition a *C*-methyl group. Of the two remaining alkaloids, virgilidine, $C_{10}H_{19}NO$, was thought to be an isomer of lupinine, and the other appeared to be a mixture of (±)-lupanine with some (+)-lupanine. The present work on the basic extract from the seeds of *V. oroboides*, a species which is reported to be identical with *V. capensis*,[2] has resulted in the isolation of a base having the same physical properties as those reported for virgiline. The principal constituent of the basic extract is an alkaloid which we named oroboidine in a preliminary communication [3] and which did not appear to be present in White's extract. After the acceptance of this communication, Goosen [4] independently reported the isolation from *Calpurnia subdecandra* of a new alkaloid, calpurnine, which we have found to be identical with oroboidine (this name is now abandoned). Of the remaining alkaloids, epilupinine is probably identical with virgilidine although there was no evidence for the occurrence of lupanine in our extract; evidence from filter-disc paper chromatography in two solvent systems suggested that 13-hydroxylupanine and cytisine might also be present.

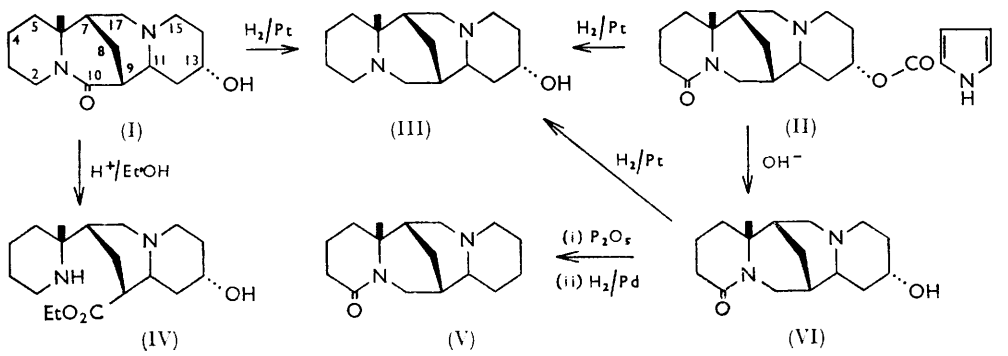
Examination of the crude chloroform extract from the seeds by paper and thin-layer chromatography in a number of solvent systems revealed the presence of five alkaloids. Virgiline (I) was separated first by using its slight solubility in acetone, and calpurnine (II) was obtained as the crystalline acetate by treatment of the mixture with dilute acetic acid. Subsequent separation of epilupinine was effected by column chromatography on alumina, and high-vacuum distillation of selected eluates. The remaining two alkaloids were not separated.

Microanalyses of virgiline and its derivatives indicate that the correct formula is $C_{15}H_{24}N_2O_2$, an isomer of 13-hydroxylupanine. It behaves as a monoacidic base and gives a monomethiodide. Titration with hydrochloric acid indicated an equivalent of 266 (molecular weight 264), and hence one of the two nitrogen atoms is non-basic. The infrared spectrum of virgiline showed peaks at 3350 and 1617 cm.$^{-1}$, indicative of a hydroxyl group and a lactam, respectively, thereby accounting for both of the oxygen atoms. Acetylation with acetic anhydride gave a mono-*O*-acetyl derivative. The key to the structure of virgiline was provided by catalytic hydrogenation in acid solution which gave (−)-13-hydroxysparteine (III), identical with an authentic specimen. It then only remained to place the lactam carbonyl, which could occupy one of four positions. Position 2 was excluded since virgiline differed from the known 13-hydroxylupanine [5,6]

[1] White, *New Zealand J. Sci. Technol.*, 1946, **27**, *B*, 478.
[2] Kew Index.
[3] Gerrans and Harley-Mason, *Chem. and Ind.*, 1963, 1433.
[4] Goosen, *J.*, 1963, 3067.
[5] Marion and Douglas, *Canad. J. Chem.*, 1951, **29**, 721.
[6] Bohlmann, Winterfeldt, Schmidt, and Reusche, *Chem. Ber.*, 1961, **94**, 1767.

and its epimer, jamaidine,[7] and position 17 was rejected since lactams in this position cannot be readily reduced (cf. 17-oxosparteine [8]). Position 15 was unlikely since this would constitute a β-hydroxy-ketone which would be readily dehydrated, whereas virgiline is resistant to dehydration. There remained only position 10, which is the location of the lactam in the alkaloid aphylline obtained from *Anabasis aphylla* (Chenopodiaceae). Galinovsky and his co-workers [9] showed that aphylline is readily susceptible to acid hydrolysis; virgiline, under the same conditions, gave an amino-acid which was characterised as its ethyl ester (IV).

Virgiline is thus (−)-13-hydroxyaphylline, and appears to be the first aphylline-type alkaloid to be found in the Leguminosae. The hydroxyl group is axial [6] (ring D in the chair form), since (−)-13-hydroxysparteine (III) is produced on catalytic hydrogenation.



The absolute configuration of (−)-virgiline can be established from an extension of the work of Okuda, Tsuda, and Kataoka,[10] who recently established the absolute configurations of many of the lupin alkaloids. (+)-Lupanine (V) has the absolute configuration shown, and is obtained from (+)-13-hydroxylupanine (VI) by dehydration with phosphorus pentoxide and catalytic hydrogenation of the unsaturated intermediate. (+)-13-Hydroxylupanine, on catalytic hydrogenation, gives (−)-13-hydroxysparteine (III) which is also obtained by reduction of (−)-virgiline. Since none of these reactions involves the asymmetric centres at C-6 and C-11 it is possible to assign to (−)-virgiline the absolute configuration (I).

Calpurnine (II), $C_{20}H_{27}N_3O_3$, comprises 40% of the basic extract, and behaves as a monoacidic base, forming a methiodide, perchlorate, and acetate. The infrared spectrum (Nujol) showed peaks at 3370 (NH), 1694 (unsaturated ester), 1622 (lactam), and 1550 cm.$^{-1}$ (conjugated double bond), and the ultraviolet spectrum had $\lambda_{max.}$ 266 mμ (ε 16,180). The absence of alcoholic OH and basic NH groups was confirmed by the failure of calpurnine to form *O*-acetyl and *N*-acetyl derivatives with acetic anhydride. Hydrolysis of calpurnine with dilute sodium hydroxide solution produced two known compounds, (+)-13-hydroxylupanine (VI) and pyrrole-2-carboxylic acid, both of which were identical with authentic samples, thereby establishing the structure of calpurnine as (II). Hydrogenation of calpurnine with Adams catalyst in dilute acid solution yielded (−)-13-hydroxysparteine (III) and proline. The 13-hydroxysparteine was identical with an authentic specimen obtained by the catalytic reduction of (+)-13-hydroxylupanine, and the presence of proline was confirmed by paper chromatography in two solvent systems, using authentic material as standard. It was not ascertained whether the hydrolysis of the proline ester occurred during the hydrogenation or when the reaction mixture was basified during the work-up.

[7] Lloyd, *J. Org. Chem.*, 1961, **26**, 2143.
[8] Galinovsky and Stern, *Ber.*, 1944, **77**, 132.
[9] Spath, Galinovsky, and Mayer, *Ber.*, 1942, **75**, 805.
[10] Okuda, Tsuda, and Kataoka, *Chem. and Ind.*, 1961, 1115.

Bohlmann, Winterfeldt, and Brackel [11] suggested from infrared studies of synthetic isomeric hydroxysparteines that (−)-13-hydroxysparteine contains an equatorial hydroxyl group, and on this basis Goosen [4] proposed that the ester group in calpurnine is also equatorial. However, in a later Paper, Bohlmann, Winterfeldt, Schmidt, and Reusche,[6] using the rates of hydrolysis of azobenzenecarboxylic acid esters as criteria, reversed this assignment. (+)-Calpurnine thus has the absolute stereochemistry shown in (II) (ester group axial).

Calpurnine is of interest in that it appears to be the first alkaloid ester of pyrrole-2-carboxylic acid and only the second derivative of this acid to be found in Nature. Kelly, Whittingham, and Wiesner [12] reported the isolation of ryanodine, a pyrrole-2-carboxylate of ryanodol. More recently Wiewiorowski and Bretek [13] isolated the first ester alkaloid in the sparteine series and showed it to be the *trans*-cinnamate of 13-hydroxylupanine, whilst Bohlmann and his co-workers [14] lately isolated the corresponding tiglic acid ester.

Of the three remaining alkaloids, which were present in very small amounts, only one was isolated in sufficient amount for structural work to be attempted. This was a white crystalline solid, m. p. 78°, $\nu_{max.}$ (Nujol) 3170 cm.$^{-1}$. Reaction with methyl iodide produced two compounds, having m. p. 245 and 265°, respectively. Epilupinine (III) has been reported [15] to have m. p. 78° and an infrared absorption at 3180 cm.$^{-1}$, whilst Crow and Riggs [16] reported the preparation and characterisation of the two stereochemically possible methiodides [17] with melting points 251 and 265°. From this evidence, and from the correlation of the R_F values in the Table, we suggest that this base is epilupinine and probably corresponds to White's virgilidine.

White [18] reported the R_F values of a number of lupin alkaloids in various solvent systems, using filter-disc paper chromatography. Application of this technique has enabled us to correlate the R_F values (see Table) of the remaining two alkaloids with two known bases, namely 13-hydroxylupanine and cytisine. It is not possible to say whether 13-hydroxylupanine is naturally occurring or an artifact, since during the extraction the seeds were dampened with concentrated ammonia, which could have hydrolysed some of the calpurnine to 13-hydroxylupanine and pyrrole-2-carboxylic acid. The small amounts of these bases, and the lack of further plant material, precluded further work.

Experimental

Extraction and Separation.—Dried, finely milled seeds (8 kg.) of *Virgilia oroboides* were dampened with concentrated ammonia and exhaustively extracted (Soxhlet) with chloroform. The chloroform was evaporated under reduced pressure to give a green-brown gum, which was dissolved in dilute hydrochloric acid (1%). Non-basic material was filtered off, washed with dilute hydrochloric acid, and discarded. The combined acid extracts were basified with aqueous ammonia and exhaustively extracted with chloroform. The extract was concentrated under reduced pressure, to yield a brown powder (163 g., 2% of plant material). The crude extract was dissolved in acetone and set aside in the refrigerator overnight. Sparingly soluble virgiline (15 g.) separated, and was filtered off and washed with acetone. The combined acetone solutions were evaporated to dryness and the resulting powder was dissolved in acetic acid (50%; 400 ml.) and set aside for 48 hr. The solution was evaporated under reduced pressure and, when the wall of the vessel was scratched, yielded a crystalline mass of calpurnine acetate (65 g.) which was filtered off and washed with acetone. The acetone was removed and the treatment with acetic acid (50%; 300 ml.) repeated, to give a second crop of the acetate (20 g.,

[11] Bohlmann, Winterfeldt, and Brackel, *Chem. Ber.*, 1958, **91**, 2194.
[12] Kelly, Whittingham, and Wiesner, *Canad. J. Chem.*, 1951, **29**, 905.
[13] Wiewiorowski and Bratek, *Bull. Acad. polon. Sci., Ser. Sci. biol.*, 1962, **10**, 349.
[14] Bohlmann, Winterfeldt, Janiak, Schumann, and Laurent, *Chem. Ber.*, 1963, **96**, 2254.
[15] Thomas, Vipond, and Marion, *Canad. J. Chem.*, 1955, **33**, 1290.
[16] Crow and Riggs, *Austral. J. Chem.*, 1955, **8**, 136.
[17] Moynehan, Schofield, Jones, and Katritzky, *J.*, 1962, 2637.
[18] White, *New Zealand J. Sci. Technol.*, 1957, **38**, *B*, 707.

total yield 85 g.). The combined filtrates were freed from acetone, dissolved in dilute hydrochloric acid (1%), basified with aqueous ammonia, and exhaustively extracted with chloroform. The solution was evaporated, and the residue was treated with acetone and set aside, to give a further crop of virgiline (2 g., total yield 17 g.). After removal of the acetone a portion of the residue (15 g.) was dissolved in dry benzene (50 ml.) and chromatographed on alumina (500 g.). The column was developed initially with benzene (1300 ml.) and subsequently with benzene containing increasing amounts of chloroform (10—60% of chloroform in benzene, 2600 ml.). Samples were collected at 100 ml. intervals and examined by thin-layer chromatography, using alumina on microscope slides and developing in chloroform. The second major fraction (2·3 g.), which was eluted with 70% chloroform in benzene, was sublimed at 70—110°/10^{-4} mm. for 30 hr., to give a mixture of oil and crystalline material (0·1 g.), identified as epilupinine. At higher temperatures calpurnine sublimed and the residue decomposed.

Examination of the Extract by Paper Chromatography.—Paper chromatograms, run with butan-1-ol–glacial acetic acid–water (4 : 1 : 5) as ascending phase, and developed with Dragendorff–Munier reagent, showed five distinct spots: R_F 0·72 (calpurnine), 0·65 (epilupinine), 0·57 (virgiline), 0·52 (possibly 13-hydroxylupanine), and 0·38 (possibly cytisine). The results of the filter-disc paper chromatograms, which were run as described by White,[18] are summarised in the Table.

R_F Values of alkaloids obtained from *V. oroboides* using filter-disc paper chromatography.

	Solvent (A)			Solvent (B)		
Alkaloid	1	2	3	1	2	3
Calpurnine	—	0·86	0·87	—	0·59	0·59
Epilupinine	0·79	0·79	0·79	0·45	0·51	0·51
Virgiline	0·65	0·61	0·61	0·37	0·37	0·37
13-Hydroxylupanine	—	0·52	0·52	—	0·34	0·35
Cytisine	0·34	0·35	—	0·19	0·19	—

Solvent (A), butanol–water–36% hydrochloric acid (50 : 17 : 7·5). Solvent (B), butanol–water–glacial acetic acid (50 : 17 : 2). 1, Values reported by White.[18] 2, Values obtained from basic extract from *V. oroboides*. 3, Values obtained from authentic specimens.

(−)-*Virgiline* (I).—*Virgiline* crystallised from acetone–methanol as prisms, m. p. 249—250° (lit.,[1] 248°), $[\alpha]_D^{20}$ −39° (*c* 1·59 in ethanol) (Found: C, 68·05; H, 9·0; N, 10·5. $C_{15}H_{24}N_2O_2$ requires C, 68·2; H, 9·2; N, 10·6%). The *methiodide* crystallised from acetone–methanol as prisms, m. p. 170—171° (lit.,[1] 173—174°) (Found: C, 47·3; H, 7·1; N, 7·2. $C_{16}H_{27}IN_2O_2$ requires C, 47·3; H, 6·7; N, 6·9%). The *O-acetyl derivative*, prepared by treatment with acetic anhydride in pyridine, crystallised from ether–acetone as prisms, m. p. 175—176° (lit.,[1] 173—174°) (Found: C, 66·7; H, 8·6; N, 9·0. $C_{17}H_{26}N_2O_3$ requires C, 66·6; H, 8·6; N, 9·1%).

(−)-13-*Hydroxysparteine* (III).—Virgiline (0·52 g.) in hydrochloric acid (2N; 20 ml.), was hydrogenated using Adams catalyst. Two molar equivalents of hydrogen were taken up in 15 hr. The catalyst was filtered off, and the filtrate basified with sodium hydroxide solution and extracted with ether. On evaporation a white solid was obtained which was crystallised from ether and sublimed at 135—140°/0·01 mm., to give (−)-13-hydroxysparteine as needles, m. p. 152°, mixed m. p. 151° (lit.,[5] 150—151°), $[\alpha]_D^{20}$ −26·5° (*c* 1·30 in ethanol) (Found: C, 72·1; H, 10·7; N, 11·2. Calc. for $C_{15}H_{26}N_2O$: C, 72·0; H, 10·5; N, 11·2%).

Ethyl Virgilate (IV).—Virgiline (0·5 g.) was refluxed for 5 hr. with hydrochloric acid (5%; 50 ml.). The solution was concentrated to give an amorphous solid which was dissolved in absolute ethanol, and the solution was evaporated to dryness. The residue was redissolved in absolute ethanol and the solution saturated with dry hydrogen chloride. After removal of the ethanol the product was shaken with a solution of potassium carbonate and extracted with ether, to give a yellow oil. *Ethyl virgilate* crystallised from light petroleum (b. p. 60—80°) as small prisms, m. p. 128° (Found: C, 65·9; H, 9·9; N, 9·0. $C_{17}H_{30}N_2O_3$ requires C, 65·8; H, 9·7; N, 9·0%), $\nu_{max.}$ 3240 (NH), 3120 (OH), and 1734 $cm.^{-1}$ (ester).

(+)-*Calpurnine* (II).—Calpurnine acetate was dissolved in water, and the solution was basified with aqueous ammonia and extracted with chloroform. On evaporation of the solvent the free base was obtained as a white powder. Recrystallisation from ethyl acetate gave prisms, m. p. 153—154° (lit.,[4] 152—154°) on seeding with a crystalline sample kindly provided

by Dr. A. Goosen. The product had $[\alpha]_D^{20}$ +47° (*c* 1·16 in ethanol) (Found: C, 67·0; H, 7·3; N, 11·7. Calc. for $C_{20}H_{27}N_3O_3$: C, 67·2; H, 7·6; N, 11·8%). The *acetate* crystallised from acetone as prisms, m. p. 144° (Found: C, 63·1; H, 7·2; N, 9·6. $C_{22}H_{31}N_3O_5$ requires C, 63·3; H, 7·5; N, 10·1%). The methiodide crystallised from acetone–methanol as small prisms, m. p. 250° (lit.,[4] 230—233°) (Found: C, 50·6; H, 6·0; N, 8·5. Calc. for $C_{21}H_{30}IN_3O_3$: C, 50·5; H, 6·1; N, 8·4%). The perchlorate crystallised from methanol as needles, m. p. 279° (lit.,[4] 255—260°) (Found: C, 52·8; H, 6·4; N, 9·0. Calc. for $C_{20}H_{28}ClN_3O_7$: C, 52·5; H, 6·2; N, 9·2%).

(+)-13-*Hydroxylupanine* (VI).—Calpurnine (2 g.) was dissolved in sodium hydroxide solution (2%; 20 ml.) containing a little ethanol, and warmed for 6 hr. The solution was extracted with chloroform and concentrated, to give (+)-13-hydroxylupanine as a gum which crystallised from acetone as prisms, m. p. 166—167° (lit.,[5] 169—170°), $[\alpha]_D^{20}$ +44° (*c* 1·34 in ethanol) (Found: C, 68·2; H, 9·4; N, 10·2. Calc. for $C_{15}H_{24}N_2O_2$: C, 68·2; H, 9·2; N, 10·6%). The alkaline solution was acidified and extracted with ether which was evaporated to give a brown solid. Crystallisation from ether gave pyrrole-2-carboxylic acid, m. p. and mixed m. p. 205—208°.

(−)-13-*Hydroxysparteine* (III).—Calpurnine (0·8 g.) in hydrochloric acid (2N; 25 ml.) was hydrogenated using Adams catalyst. Four molar equivalents of hydrogen were taken up in 24 hr., and the solution was worked up as for virgiline, to give (−)-13-hydroxysparteine, m. p. 152° (lit.,[5] 150—151°) (Found: C, 71·7; H, 10·4; N, 11·0. Calc. for $C_{15}H_{26}N_2O$: C, 72·1; H, 10·5; N, 11·2%). The presence of proline in the solution after extraction of the 13-hydroxysparteine was confirmed by paper chromatography in two solvent systems [phenol–water (4 : 1) containing a trace of hydrogen cyanide; and propan-1-ol–water (3 : 1)], using authentic proline as reference.

Epilupinine.—Epilupinine, crystallised from light petroleum (b. p. 40—60°), had m. p. 78°, $\nu_{max.}$ 3170 cm.$^{-1}$. The α-methiodide, prepared by refluxing epilupinine in acetone with an excess of methyl iodide for 2 hr., crystallised from acetone as prisms, m. p. 245° (lit.,[16,19] 251°, 247°). The β-methiodide, prepared by warming epilupinine with an excess of methyl iodide in acetone, crystallised from acetone as prisms, m. p. 264—265° (lit.,[16] 265°) (Found: C, 43·0; H, 7·1; N, 4·3. Calc. for $C_{11}H_{22}INO$: C, 42·5; H, 7·1; N, 4·5%).

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[19] White, *New Zealand J. Sci. Technol.*, 1951, **33**, *B*, 50.