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BIOSYNTHESIS OF DIOSCORINE

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Key Word Index—*Dioscorea hispida*; Dioscoreaceae; dioscorine; alkaloid biosynthesis; [1-^{14}C]-acetate; [6-^{14}C]-Δ^1-piperideine; oxalic acid biosynthesis.

Abstract—The administration of [1-^{14}C]-acetate of *Dioscorea hispida* plants afforded radioactive dioscorine (0·2% incorporation). A systematic degradation established that the bulk of the activity was located at alternate carbons of the unsaturated lactone ring (C-5: 31%, C-10: 28%, C-12: 28%). [2-^{14}C]-lysine, a plausible precursor of part of the isoquinuclidine nucleus, did not afford dioscorine containing a significant amount of activity. Dioscorine obtained from plants which had been fed [6-^{14}C]-Δ^1-piperideine had low activity (0·03% incorporation). However, a partial degradation indicated that the alkaloid was not labelled at one specific position, and the pattern of labelling was consistent with catabolism of the Δ^1 -piperideine to acetate, prior to its incorporation into dioscorine.

INTRODUCTION

DIOSCORINE (I)[1,2] and dihydrodioscorine (IV) are the only known alkaloids which contain an isolated isoquinuclidine nucleus. Catharanthine[3] and other indole alkaloids of the *Iboga* type and cannivonine, recently isolated from cranberry leaves,[4] contain the isoquinuclidine nucleus, but in these alkaloids it is part of a condensed ring system. We suggested in 1967[5] that the biosynthesis of dioscorine involved six acetate units which formed a branched chain as indicated in Scheme 1 (Route A). An alternate biosynthetic pathway (Route B) involves the condensation of Δ^1-piperideine (II) (which is known to be formed from lysine in other higher plants[6] with an eight-carbon unit derived from four acetate units. Spenser[7] suggested a third scheme (Route C), involving the condensation of pelletierine (III) with a four-carbon unit.

* Contribution No. 119 from this Laboratory.

[1] See C. B. PAGE and A. R. PINDER, *J. Chem. Soc.* 4811 (1964); and references cited therein for the synthesis and ultimate structure determination of dioscorine.

[2] Dioscorine, often accompanied with its dihydro derivative has been isolated from many species of *Dioscorea*: see J. J. WILLAMAN and B. G. SCHUBERT, Alkaloid-Bearing Plants and Their Contained Alkaloids. *Tech. Bull.* No. 1234, Agricultural Research Service, U.S. Dept. of Agriculture (1961); J. J. WILLAMAN and H.-L. LI, *Lloydia* **33**, Supplement No. 3A (1970).

[3] G. H. SVOBODA, I. S. JOHNSON, M. GORMAN and N. NEUSS, *J. Pharm. Sci.* **51**, 707 (1962).

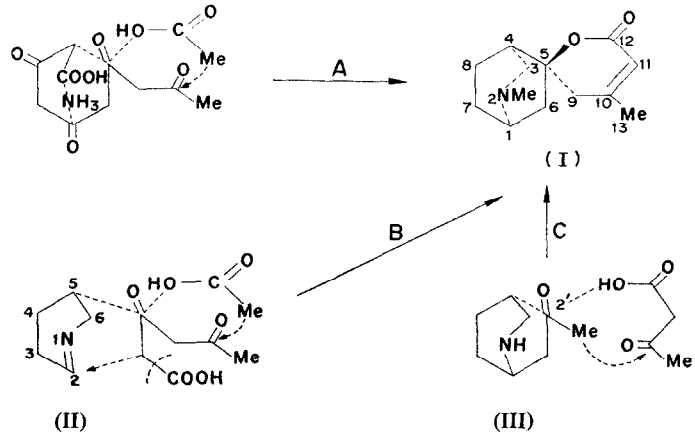
[4] K. JANKOWSKI and I. JANKOWSKI, *Experientia* **27**, 1383 (1971).

[5] E. LEETE, in *Biogenesis of Natural Compounds*, 2nd edition (edited by P. BERNFELD), p. 968, Pergamon Press, Oxford (1967).

[6] E. LEETE, *J. Am. Chem. Soc.* **91**, 1697 (1969).

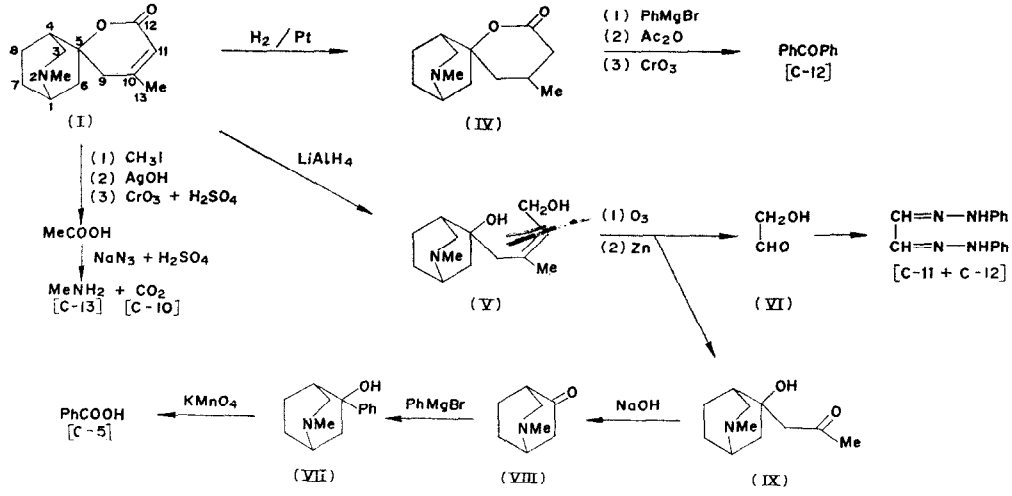
[7] J.-C. BRAEKMAN, R. N. GUPTA, D. B. MCLEAN and I. D. SPENSER, *Can. J. Chem.* in press.

In order to examine these hypotheses various potential precursors were fed to the tropical yam, *Dioscorea hispida*, Dennst. In preliminary studies[8] sodium [1-^{14}C]- and [2-^{14}C]-acetate, [2-^{14}C]-lysine and [1-^{14}C]-senecioic acid (a plausible precursor of the unsaturated lactone ring) were administered to the yam, either by the wick method or by direct injection into the tubers (the main source of dioscorine). In none of these experiments was any significant amount of radioactivity detected in the dioscorine. Radioactive dioscorine



SCHEME 1. HYPOTHETICAL BIOSYNTHETIC ROUTES TO DIOSCORINE.

(0·2% incorporation) was ultimately obtained by spraying the leaves of the yam with an aqueous solution of sodium [1-^{14}C]-acetate.[9] Scheme 2 illustrates the various degradations carried out to determine the distribution of activity in the labelled dioscorine. A Kuhn–Roth oxidation on dioscorine methohydroxide afforded acetic acid, which was subjected

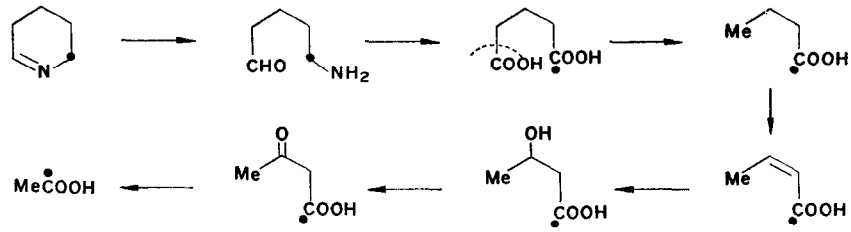


SCHEME 2. DEGRADATION OF RADIOACTIVE DIOSCORINE.

[8] See C. B. PAGE, Ph.D. dissertation, University College of Cardiff, Wales (1965), for early attempts to obtain incorporation of tracers into dioscorine.

[9] Preliminary communication: E. LEETE and A. R. PINDER, *Chem. Commun.* 1499 (1971).

to a Schmidt reaction yielding methylamine, assayed as its *N*-benzoyl derivative, and carbon dioxide collected as barium carbonate. Dihydrodioscorine (IV) was subjected to a Barbier–Wieland degradation affording C-12 as benzophenone, assayed as its oxime. Reduction of dioscorine with $LiAlH_4$ yielded dioscorinol (V),[10] which on ozonolysis and treatment with zinc afforded 5-acetonyl-5-hydroxy-2-methylisoquinuclidine (IX)[11] and glycollic aldehyde (VI) isolated as its osazone. The acetonyl derivative IX was cleaved with dilute NaOH yielding 2-methyl-5-oxoisoquinuclidine (VIII).[12] Reaction of this ketone with phenyl magnesium bromide yielded 5-hydroxy-2-methyl-5-phenylisoquinuclidine (VII),[13] which was oxidized with $KMnO_4$ affording benzoic acid, representing the activity at C-5. The degradation was carried out in duplicate and the average activities are recorded in Table 1. It is clear that the dioscorine derived from [1-^{14}C]-acetate had approximately one-third of its activity located at C-5, C-10 and C-12, a result which is not consistent with the formation of the alkaloid from six acetate units (Route A). However, biosynthetic Routes B and C are compatible with our experimental results. In scheme C involving pelletierine, the propyl side chain has to be labelled at C-2′. This pattern of labelling was in fact observed in the pelletierine isolated from *Punica granatum* plants which had been fed [1-^{14}C]-acetate.[14]



SCHEME 3. HYPOTHETICAL CATABOLISM OF [6-^{14}C]-Δ^1-PIPERIDEINE TO [1-^{14}C]-ACETATE.

Since we obtained a successful incorporation of [1-^{14}C]-acetate by topical application to the leaves of the yam, [2-^{14}C]-lysine and [6-^{14}C]-Δ^1-piperideine[6] were fed by the same method. The dioscorine isolated from the plants which had been fed the lysine had very low activity (0·003% incorporation). Dioscorine obtained from plants which had been fed the Δ^1-piperideine contained a significant amount of activity (0·03% incorporation). If this compound had been incorporated in accordance with Route B all the activity of the dioscorine would be located at C-3. On carrying out a partial degradation (see Table 1) it became apparent that the Δ^1-piperideine had not been incorporated specifically. Indeed, the pattern of labelling was essentially the same as that in the dioscorine derived from [1-^{14}C]-acetate. It is thus suggested that the [6-^{14}C]-Δ^1-piperideine undergoes catabolism affording [1-^{14}C]-acetate, plausibly as illustrated in Scheme 3. The origin of that part of dioscorine not derived from acetate thus remains unknown. We consider that this moiety

[10] A. R. PINDER, *Tetrahedron* **1**, 301 (1957).
[11] J. B. JONES and A. R. PINDER, *J. Chem. Soc.* 615 (1959).
[12] W. A. M. DAVIS, J. B. JONES and A. R. PINDER, *J. Chem. Soc.* 3504 (1960). The product of this retroactive aldol condensation was initially thought to be tropan-2-one, but was later revised to structure VIII [I. G. MORRIS and A. R. PINDER, *J. Chem. Soc.* 1841 (1963)].
[13] The relative positions of the phenyl and hydroxy groups in this compound were not determined, however the sharp m.p. of the isolated product probably indicates that only one isomer was obtained on phenylation of the ketone.
[14] M. F. KEOGH and D. G. O'DONOVAN, *J. Chem. Soc.*, C, 1792 (1970).

could be formed from lysine, and we rationalize the lack of incorporation of this amino acid or Δ^1-piperideine by suggesting that the plant contains, at the time we have carried out feeding, some compound (derivable from lysine or other precursor) which is not being actively synthesized, but is available for condensation with acetate to yield dioscorine.

Although not relevant to the present investigation it should be reported that radioactive oxalic acid (0·04% incorporation) was isolated from the *Dioscorea* plants which had been fed [1-^{14}C]-acetate. Oxalic acid is widely distributed in plants, and Krebs cycle intermediates have been shown to be its precursor in *Oxalis pes-caprae*.[15]

TABLE 1. ACTIVITY OF DIOSCORINE AND ITS DEGRADATION PRODUCTS

Compound	From [1-^{14}C]-acetate Specific activity (dpm/mmol $\times 10^{-6}$)	Relative specific activity	From [6-^{14}C]-Δ^1-piperideine Specific activity (dpm/mmol $\times 10^{-4}$)	Relative specific activity
Dioscorine picrate	9·95	100	5·9	100
Dioscorine methiodide	10·1	101		
1-Acetamidonaphthalene	3·14	32 ± 1		
N-Methylbenzamide [C-13]	0·02	0·02		
Barium carbonate [C-10]	2·76	28 ± 2		
Dihydrodiocorine picrate	10·2	102		
Benzophenone oxime [C-12]	2·8	28 ± 1		
Dioscorinol picrate	9·90	100	5·85	99
Dioscorinol picrolonate	9·85	99		
Glyoxal bisphenylhydrazone [C-11 + C-12]	3·2	32 ± 2	2·0	34
5-Acetonyl-5-hydroxy-2-methylisoquinuclidine	6·43	65 ± 1		
2-Methyl-5-oxoisoquinuclidine picrate	3·65	36 ± 1	2·2	37
5-Hydroxy-2-methyl-5-phenylisoquinuclidine	3·6	36 ± 2	2·1	36
Benzoic acid [C-5]	3·05	31 ± 1	1·7	29

EXPERIMENTAL

General methods. Radioactive compounds were assayed in duplicate in a Nuclear Chicago Mark II liquid scintillation counter, using as solvents either dioxane–EtOH or toluene, with the usual scintillators.[16] Microanalyses were determined by Clark microanalytical laboratories, Urbana, Illinois.

Administration of tracers to Dioscorea hispida *and isolation of the dioscorine.* Sodium [1-^{14}C]-acetate (New England Nuclear, Boston, Mass) (41 mg, 1·0 mCi) was dissolved in H_2O (20 ml) and sprayed (using a chromatographic sprayer) onto the large leaves of two *D. hispida* plants growing in soil in a greenhouse (July 1971, Minnesota). The leaves were supported in a horizontal position by means of thick copper wire. On subsequent days the plants were watered by spraying the leaves with distilled water. After 14 days the plants were harvested (wet wt 420 g) and macerated in a Waring Blendor with 0·5 N HCl (2 l.) with cooling. After standing for 2 days the mixture was filtered and the pale yellow filtrate ($2·6 \times 10^8$ dpm = 12% of the activity fed) made alkaline with K_2CO_3 and extracted continuously with Et_2O for 4 days. The dried ($MgSO_4$) extract was evaporated and the residue distilled (140°, 0·001 mm) affording crude dioscorine (100 mg). TLC on Silica gel G (Merck) developing with $CHCl_3$–EtOH–conc. NH_4OH (100:10:0·5) indicated that essentially all the activity was located at a spot coincident with dioscorine (R_f 0·3). The dioscorine was converted to its picrate, m.p. 183–184°, and crystallized to constant activity from Me_2CO–EtOAc. The activity of this material and the degradation products of dioscorine are recorded in Table 1. The alkaline solution from which the dioscorine had been extracted was acidified with HCl and extracted with Et_2O for 7

[15] A. MILLERD, R. K. MORTON and J. R. E. WELLS, *Biochem. J.* **86**, 57 (1963).
[16] A. R. FREIDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).

days. Evaporation of the extract yielded a sticky solid which was crystallized from $CHCl_3$–Et_2O, affording a white solid which was sublimed (100°, 0·01 mm). Recrystallization from Me_2CO–$CHCl_3$ yielded colorless needles (158 mg, 5200 dpm/mg), m.p. 104–105°, identical (m.m.p., IR) with an authentic specimen of oxalic acid. In a preliminary feeding experiment in which a solution of sodium [1-^{14}C]-acetate (20·5 mg, 0·5 mC) was painted on the leaves of *D. hispida* plants (June, 1971) radioactive dioscorine (90 mg, $1{\cdot}7 \times 10^6$ dpm/mmol) was obtained, representing an incorporation of 0·06%. DL-[2-^{14}C]-Lysine monohydrochloride 19·9 mg, 0·25 mC) was fed to two *D. hispida* plants (August 1971) which yielded after 14 days dioscorine (79 mg, $4{\cdot}5 \times 10^4$ dpm/mmol = 0·003% incorporation). [6-^{14}C]-Δ^1-Piperideine hydrochloride[6] (0·55 mmol, 0·1 mC) was fed to four plants (September 1971) which yielded after 14 days dioscorine (225 mg, $5{\cdot}9 \times 10^4$ dpm/mmol = 0·03% incorporation).

Degradation of the dioscorine. Dioscorine was liberated from its picrate by suspending in 2 N HCl, extracting the picric acid with Et_2O, and then Et_2O extraction of the aqueous solution which had been made alkaline with K_2CO_3 yielded dioscorine.

Kuhn–Roth oxidation on dioscorine methiodide. Dioscorine (156 mg) was dissolved in MeOH (10 ml) and MeI (1 ml) added. After standing overnight, evaporation yielded a colorless residue which was crystallized from EtOH–EtOAc affording dioscorine methiodide (234 mg) m.p. 214–216° (lit.[17] 217°). The methiodide (200 mg) was dissolved in H_2O (5 ml) and shaken with AgOH (from 0·5 g of $AgNO_3$). The mixture was filtered and the filtrate added to a solution of CrO_3 (5 g) in 2 N H_2SO_4 (50 ml). The solution was distilled and the distillate titrated with NaOH. The residue obtained on evaporation was crystallized from EtOH–Et_2O yielding sodium acetate (22 mg). For assay a portion was converted to 1-acetamidonaphthalene.[18]

Barbier–Wieland degradation on dihydrodioscorine. Dioscorine (253 mg) was dissolved in EtOH (30 ml) and hydrogenated in the presence of PtO_2 (0·1 g) at 2 atmospheres pressure for 5 hr. The filtered mixture was evaporated and the residue distilled (130°, 0·01 mm) yielding dihydrodioscorine, which yielded a picrate, m.p. 167–168°. The dihydrodioscorine (200 mg) dissolved in Et_2O (10 ml) was added slowly to a solution of phenyl magnesium bromide (from Mg (0·36 g), bromobenzene (2·33 g) and Et_2O (10 ml)). Benzene (100 ml) was then added and the mixture distilled until the b.p. reached 75°. After refluxing for 18 hr the mixture was cooled and extracted with 2 N HCl (3×30 ml). The combined HCl extracts were washed with Et_2O and then neutralized with NaOH, and extracted with $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ extract was evaporated and the residue dissolved in a mixture of pyridine (1 ml) and Ac_2O (1 ml) and heated at 100° for 1 hr. The mixture was evaporated and the residue refluxed with acetic acid (10 ml) for 18 hr. The solution was evaporated, the residue made basic with NaOH, and then extracted with $CHCl_3$. The dried ($MgSO_4$) extract was evaporated and the residue distilled (200°, 0·01 mm) affording a colorless viscous oil (135 mg). This material was dissolved in HOAc (10 ml) and mixed with a solution of CrO_3 (0·5 g) in H_2O (1 ml) and HOAc (5 ml). After stirring the mixture at 60° for 12 hr, a little MeOH was added to destroy the excess CrO_3. H_2O was added and the mixture extracted with Et_2O. The Et_2O was washed with H_2O and then with saturated $NaHCO_3$ and dried over $MgSO_4$. The residue obtained on evaporation was distilled into a U-tube cooled to −80°. The contents of the tube were boiled with a solution of NH_2OH·HCl (100 mg) in EtOH (5 ml) and aqueous 10% NaOH (1 ml) for 1 hr. The EtOH was then evaporated and the solution neutralized with HOAc, when benzophenone oxime (7 mg) m.p. 144°, separated.

Dioscorinol.[10] Dioscorine (589 mg) was dissolved in Et_2O (100 ml) and stirred at room temperature with $LiAlH_4$ (0·4 g) for 16 hr. H_2O and celite were then added and the mixture filtered. The dried (K_2CO_3) Et_2O layer was evaporated and the residue distilled (150°, 0·001 mm) affording dioscorinol (486 mg), which was assayed as its picrate, m.p. 161–162°, and picrolonate, m.p. 212–213°.

5-Acetonyl-5-hydroxy-3-methylisoquinuclidine (IX). Dioscorinol 242 mg) was dissolved in HOAc (10 ml), cooled to 10°, and O_3 passed through the solution for 4 hr. Et_2O (20 ml) was then added, followed by Zn dust (2 g) and the mixture stirred for 2 hr. The filtered mixture was evaporated and the residue made basic with aqueous K_2CO_3, and extracted with Et_2O overnight. The Et_2O extract was evaporated (without drying) and the residue distilled (110°, 0·01 mm) affording the acetonyl derivative as a colorless oil (104 mg). The aqueous alkaline solution which had been extracted with Et_2O was acidified with HOAc and a solution of phenyl hydrazine (0·5 ml) in HOAc added. On warming on a steam bath a brown solid separated (38 mg), which was crystallized from aq. EtOH (charcoal) affording glyoxal bisphenylhydrazone, m.p. 177–178°, as yellow plates, identical with an authentic specimen.

2-Methyl-5-oxoisoquinuclidine (VIII). The acetonyl derivative IX (98 mg) was dissolved in 0·1 N NaOH (30 ml) and allowed to stand at room temp. overnight. The solution was saturated with K_2CO_3 and extracted with Et_2O for 24 hr. The residue obtained on evaporation was distilled (100°, 0·1 mm), collecting the distillate in a U-tube cooled in dry ice. The contents of the U-tube were washed with MeOH into a solution of picric acid (120 mg) in MeOH. Pale yellow needles of (+)-2-methyl-5-oxoisoquinuclidine picrate separated (88 mg) m.p. 197° dec. (lit.[12] m.p. 188° dec.).

5-Hydroxy-2-methyl-5-phenylisoquinuclidine (VII). The picrate of VIII was diluted 10 × with synthetic racemic material[1] prior to reaction with phenyl magnesium bromide. The (±)-picrate (182 mg) was suspen-

[17] A. R. PINDER, *J. Chem. Soc.* 2236 (1952).
[18] E. LEETE, H. GREGORY and E. G. GROS, *J. Am. Chem. Soc.* **87**, 3475 (1965).

ded in 50% KOH, and extracted several times with Et_2O. The dried ($MgSO_4$) extract was evaporated and the residue, dissolved in a little Et_2O, was added to a solution of phenyl magnesium bromide (from Mg (0·36 g), bromobenzene (2·33 g) and Et_2O (10 ml)) and stirred overnight at room temp. The mixture was then treated with 2 N HCl, and the Et_2O layer extracted several times with additional HCl. The acid extract was made basic with NaOH and extracted overnight with Et_2O. The residue obtained on evaporation of the Et_2O was sublimed (120°, 0·001 mm), affording a white sublimate (54 mg) which was crystallized from light petrol. (b.p. 30–60°) yielding colorless plates of 5-hydroxy-2-methyl-5-phenyl-isoquinuclidine, m.p. 129–130°. *Anal.* Calc. for $C_{14}H_{19}NO$: C, 77·38, H, 8·81; N, 6·45. Found: C, 77·33; H, 9·11; N, 6·35%. This phenyl derivative (50 mg) was added to H_2O (50 ml) containing NaOH (0·2 g) and $KMnO_4$ (0·4 g) and the mixture refluxed for 20 hr. SO_2 was passed through the reaction mixture until a clear solution was obtained. Extraction of this solution with Et_2O afforded benzoic acid, purified by sublimation and crystallization from hot H_2O, yield 27 mg.

Acknowledgements—We thank Mr. A. G. Kenyon, Tropical Products Institute, London, who obtained the *Dioscorea hispida* tubers from Malaya. This investigation was supported by a research grant GM-13246 from the U.S. Public Health Service.