Phytochemistry, 1969, Vol. 8, pp. 1003 to 1007. Pergamon Press. Printed in England.

OBSERVATIONS ON THE ADMINISTRATION OF IRIDODIAL-7-^{14}C TO *VINCA ROSEA*[1]

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(*Received* 22 *November* 1968, *in revised form* 11 *January* 1969)

Abstract—It has been suggested that iridodial is a biosynthetic intermediate between geraniol and loganin, which is a precursor of the non-tryptophan derived portion of vindoline, catharanthine, ajmalicine, and other indole alkaloids found in *Vinca rosea*. Iridodial-7-^{14}C was synthesized from ethyl iodide-1-^{14}C and fed to *V. rosea* plants, which afforded vindoline having negligible activity. It thus seems unlikely that iridodial is a precursor of loganin.

GERANIOL (I) is an established precursor of the non-tryptophan derived nine- or ten-carbon unit which is found in a large number of indole alkaloids.[2–5] The administration of geraniol-2- or -3-^{14}C to *Vinca rosea* plants yielded labelled alkaloids, the location of the ^{14}C being consistent with a hypothesis which was suggested independently by Wenkert[6] and Thomas[7] in 1961, and is illustrated schematically in Fig. 1. There was no significant difference in a degree of incorporation of geraniol and its isomer nerol, having a *cis*-2,3 double bond.[8] Battersby[2] administered geraniol as its pyrophosphate; however, geraniol emulsified with *Tween*, a non-ionic surfactant, was also efficiently incorporated into the alkaloids.[4,5] An essential feature of the hypothesis of Thomas and Wenkert was the formation of a cyclopentanomonoterpene (II), which subsequently underwent cleavage to afford the *Corynanthe* unit (III), so-called because it occurs in corynantheine and related alkaloids. Battersby tested several natural cyclopentanomonoterpenes, labelled with tritium, as precursors of the *V. rosea* alkaloids[9]; however, he found that only loganin (XII) was an efficient precursor. The presence of loganin in *V. rosea* plants was also established.[9] More recently it has been shown that loganin, labelled with ^{14}C at specific positions, is incorporated into the *Vinca* alkaloids, in accordance with the scheme shown in Fig. 1.[10,11] Rearrangement of the *Corynanthe* unit to the ten-carbon unit found in vindoline [the *Aspidosperma* unit (IV)] and

[1] Part II in a series entitled "Biosynthesis of the *Vinca* Alkaloids"; for Part I, see E. LEETE, A. AHMAD and I. KOMPIS, *J. Am. Chem. Soc.* **87**, 4168 (1965).

[2] A. R. BATTERSBY, R. T. BROWN, J. A. KNIGHT, J. A. MARTIN and A. O. PLUNKETT, *Chem. Commun.* 346 (1966).

[3] P. LOEW, H. GOEGGEL and D. ARIGONI, *Chem. Commun.* 347 (1966).

[4] (a) E. S. HALL, F. MCCAPRA, T. MONEY, K. FUKUMOTO, T. R. HANSON, B. S. MOOTOO, G. T. PHILIPS and A. I. SCOTT, *Chem. Commun.* 348 (1966); (b) T. MONEY, I. G. WRIGHT, F. MCCAPRA, E. S. HALL and A. I. SCOTT, *J. Am. Chem. Soc.* **90**, 4144 (1968).

[5] E. LEETE and S. UEDA, *Tetrahedron Letters* 4915 (1966).

[6] E. WENKERT, *J. Am. Chem. Soc.* **84**, 98 (1962).

[7] A. F. THOMAS, *Tetrahedron Letters* 544 (1961).

[8] A. R. BATTERSBY, R. T. BROWN, R. S. KAPIL, J. A. KNIGHT, J. A. MARTIN, and A. O. PLUNKETT, *Chem. Commun.* 888 (1966).

[9] A. R. BATTERSBY, R. T. BROWN, R. A. KAPIL, J. A. MARTIN and A. O. PLUNKETT, *Chem. Commun.* 890 (1966).

[10] A. R. BATTERSBY, R. S. KAPIL, J. A. MARTIN and L. MO, *Chem. Commun.* 133 (1968).

[11] P. LOEW and D. ARIGONI, *Chem. Commun.* 137 (1968).

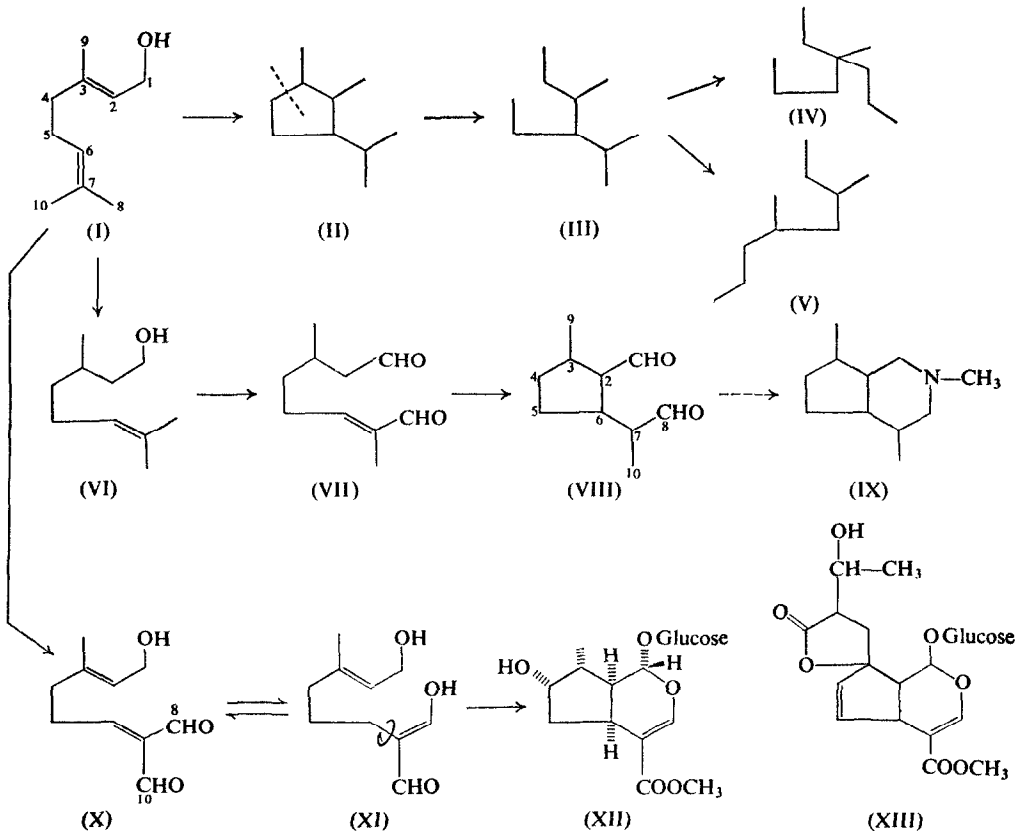

FIG. 1. HYPOTHETICAL BIOSYNTHETIC ROUTES FROM GERANIOL TO LOGANIN.

catharanthine [the *Iboga* unit (V)] probably occurs after reaction of the *Corynanthe* unit with tryptophan,[12–14] as suggested originally by Wenkert.[15] Nothing is known of the biosynthetic route from geraniol to loganin. Battersby[16] suggested a route via citronellol (VI), the dialdehyde (VII), and iridodial (VIII), which has been numbered to illustrate its biogenetic relationship to geraniol. Iridodial is a natural product[17] and a potential precursor of alkaloids such as skytanthine (IX).[18] In order to test this hypothesis we have synthesized iridodial-7-^{14}C by the route illustrated in Fig. 2. Oxidation of citronellol (XIV) yielded citronellal (XV), which was converted to its acetal (XVI). Ozonolysis yielded 4-methyl-6,6-ethylenedioxyhexanal (XIX). Reaction of this aldehyde with α-formylethylidene-1-^{14}C-triphenylphos-

[12] A. A. QURESHI and A. I. SCOTT, *Chem. Commun.* 948 (1968).
[13] A. R. BATTERSBY, J. C. BYRNE, R. S. KAPIL, J. A. MARTIN, T. G. PAYNE, D. ARIGONI and P. LOEW, *Chem. Commun.* 951 (1968).
[14] J. P. KUTNEY, C. EHRET, V. R. NELSON and D. C. WIGFIELD, *J. Am. Chem. Soc.* **90**, 5929 (1968).
[15] E. WENKERT and B. WICKBERG, *J. Am. Chem. Soc.* **1580**, 5810 (1965).
[16] A. R. BATTERSBY, *Pure Appl. Chem.* **14**, 117 (1967).
[17] G. W. K. CAVILL, *Rev. Pure Appl. Chem.* **10**, 169 (1960).
[18] H. AUDA, H. R. JUNEJA, E. J. EISENBRAUN, G. R. WALLER, W. R. KAYS and H. H. APPEL, *J. Am. Chem. Soc.* **89**, 2476 (1967).

phorane (XXII) afforded 2,6-dimethyl-8,8-ethylenedioxy-2-octenal-2-^{14}C (XVIII). The Wittig reagent (XXII) was obtained by the action of butyl lithium and ethyl formate on ethyl-1-^{14}C-triphenylphosphonium iodide (XXI),[19] which was made from triphenylphosphine and ethyl iodide-1-^{14}C. The acetal (XVIII) was identical with material obtained by the direct oxidation of XVI with SeO_2.[20] Robinson and co-workers[20] reported that the hydrolysis of the acetal (XVIII) with 50% acetic acid yielded a mixture of the dialdehyde (XVIII) and iridodial. We found that hydrolysis with 50% formic acid led to a significant improvement in the yield of iridodial.

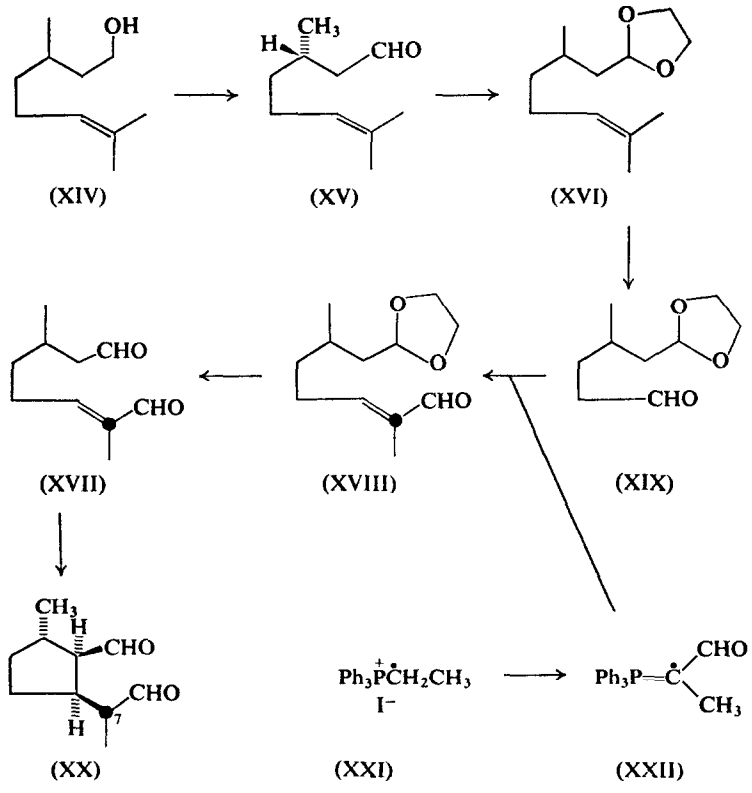

FIG. 2. SYNTHESIS OF IRIDODIAL-7-^{14}C.

The citronellal used in this synthesis contained 71 per cent of the L-isomer having the absolute configuration illustrated in formula XV.[21] Cyclization of the dialdehyde (XVII) yields iridodial having a *cis–trans* arrangement of the substituents on the cyclopentane ring[20] as illustrated in formula XX. Thus the iridodial derived from the L-citronellal will have the same absolute stereochemistry as loganin (XII).[22,23]

[19] S. TRIPPETT and D. M. WALKER, *J. Chem. Soc.* 1266 (1961).
[20] K. G. CLARK, G. I. FRAY, R. H. JAEGER and R. ROBINSON, *Tetrahedron* **6**, 217 (1959).
[21] A. BIRCH, *Ann. Rep. Chem. Soc.* **47**, 192 (1950).
[22] A. R. BATTERSBY, R. S. KAPIL and R. SOUTHGATE, *Chem. Commun.* 131 (1968).
[23] S. BRECHBÜHLER-BADER, C. J. COSCIA, P. LOEW, CH. VON SZCZEPANSKI and D. ARIGONI, *Chem. Commun.* 136 (1968).

The iridodial-7-^{14}C was emulsified in water by means of *Tween* 80, and excised shoots of *V. rosea* plants placed into this solution. The tracer was absorbed by the shoots which were harvested after 16 days. The vindoline isolated from the plants had very low activity, the actual incorporation of ^{14}C into vindoline was only 0·0005 per cent, which is considered insignificant compared with the incorporation of radioactive geraniol, administered by the same method.[24] We thus favour a route from geraniol to loganin via the dialdehyde (X) (Fig. 1). Enolization of this dialdehyde to XI would make the two aldehyde carbons radiochemically equivalent. This suggestion was also made by Schmid[25] to rationalize his results on the biosynthesis of plumieride (XIII). The pattern of labelling found in plumieride and the ubiquitous ten-carbon unit present in the indole alkaloids, after feeding mevalonic acid-2-^{14}C, requires that these two positions (C-8 and C-10) do become equivalent as some stage in the biosynthetic sequence.[4b,8 26,27]

It is possible to speculate on intermediates between the dialdehyde (X) and loganin; however, we will resist the temptation to do this and endeavour to discover the actual intermediates by experimental work.

EXPERIMENTAL

General Methods

Gas chromatography was carried out in a Wilkins Aerograph, Model 200, employing a 5 ft × ⅛ in. column, containing a cyanosilicone oil (XF-1150) absorbed on Gas Chrome Q. Optical rotations were determined in a Rudolf polarimeter, Model 80. I.r. spectra were obtained on a Unicam SP-200 spectrophotometer. Elementary analyses were carried out by Mr. J. Canterbury at the University of Minnesota. Mass spectra were determined on an Hitachi–Perkin–Elmer Model RMU-6D mass spectrometer. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation system, Model 724, using either toluene or dioxane as solvents with the usual scintillators.[28]

Citronellal (XV)

The citronellol used in this synthetic sequence had an optical rotation $[\alpha]_D^{22}$ −1·97°, whereas the reported value for pure L-citronellol is −4·76°.[29] The sample used was thus a mixture of 71 per cent L-citronellol and 29 per cent D-citronellol. Oxidation with a mixture of CrO_3 and pyridine using the method of Holman[30] afforded citronellal (49 per cent yield).

2,6-*Dimethyl*-8,8-*ethylenedioxy*-2-*octene* (*XVI*)

The citronellal was converted to its cyclic acetal by refluxing with ethylene glycol, benzene, and *p*-toluene sulphonic acid, as described by Robinson.[20] The rotation, $[\alpha]_D^{21}$ −1·65, again indicated that there was about 70 per cent of the L-isomer, lit.[20] −3·9°.

4-*Methyl*-6,6-*ethylenedioxyhexanal* (*XIX*)

The above acetal (10·4 g) was dissolved in ethyl acetate (100 ml) and cooled to −30°. O_3 was passed through this solution until all the acetal had been converted to its ozonide (the reaction was monitored by gas chromatography). Traces of O_3 were then swept out of the reaction mixture by means of N_2, and then Adams catalyst (30 mg) added. The solution was hydrogenated at 0° and 1 atmosphere pressure for 4 hr when H_2 uptake ceased. The filtered solution was evaporated and the residual oil shaken with a solution of Na_2SO_3 (24 g) and $NaHCO_3$ (27 g) in water (250 ml). The aqueous solution was extracted with ether (3 × 30 ml) and then made basic with 10 per cent NaOH. The aldehyde was extracted with ether (5 × 30 ml) which was dried over

[24] On administering geraniol-^{14}C to *V. rosea* plants we[5] obtained an incorporation of 0·04 per cent into vindoline, Scott[4b] 0·01 per cent, Arigoni[3] 0·1 per cent, and Battersby[2] administering geraniol-2-^{14}C as its pyrophosphate 0·2 per cent.

[25] D. A. YEOWELL and H. SCHMID, *Experientia* **20**, 250 (1964).

[26] H. GOEGGEL and D. ARIGONI, *Chem. Commun.* 538 (1965).

[27] A. R. BATTERSBY, R. T. BROWN, R. S. KAPIL, A. O. PLUNKETT and J. B. TAYLOR, *Chem. Commun.* 46 (1966).

[28] A. R. FRIEDMAN and E. LEETE, *J. Am. Chem. Soc.* **85**, 2141 (1963).

[29] R. RIENÄCHER and G. OHLOFF, *Angew. Chem.* **73**, 240 (1961).

[30] J. R. HOLMAN, *J. Org. Chem.* **26**, 4814 (1961).

$MgSO_4$. The residue obtained on evaporation was distilled affording 4-methyl-6,6-ethylenedioxyhexanal (2·8 g, 31 per cent), b.p. 105–110° (5 mm). Gas chromatography indicated that the product was homogeneous. $[\alpha]_D^{21}$ −0·49°, n_D^{24} 1·4585. The i.r. spectrum (liquid film) had a strong absorption at 1720 cm^{-1} (C=O). *Anal.* Calc. for $C_9H_{12}O_3$: C, 62·75; H 9·38. Found: C, 62·53; H, 9·45 per cent.

Ethyl-1-^{14}C-triphenylphosphonium Iodide (XXI)

Ethyl iodide-1-^{14}C (1·70 g, 0·40 mc)[31] and triphenylphosphine (2·9 g) were heated together in a sealed tube at 120° for 24 hr. The reaction product was crystallized from a mixture of ethanol and ether yielding colourless prisms of ethyl-1-^{14}C-triphenylphosphonium iodide (4·10 g, 90 per cent), m.p. 171–173°, lit.[19] 169–171°, having a specific activity of $7·73 \times 10^7$ dpm/mM.

α-Formylethylidene-1-^{14}C-triphenylphosphorane (XXII)

The previously described phosphonium iodide (4·1 g) was suspended in dry ether (50 ml) and a solution of butyl lithium in hexane (1·6 M, 6·8 ml) added slowly to the stirred mixture in N_2. After 10 min the red solution of the ylid was added to a well-stirred solution of ethyl formate (1·0 g) in ether (25 ml). After 10 min the resultant thick suspension was added to 2 N HCl (200 ml), and extracted with $CHCl_3$ (4 × 40 ml). The $CHCl_3$ extract was washed with 3 N Na_2CO_3 (3 × 50 ml), water (50 ml), and then dried over $MgSO_4$. The solid residue (m.p. 150–195°) obtained on evaporation was extracted with boiling benzene (3 × 50 ml.) Concentration of the benzene extract to 30 ml and dilution with petroleum ether (b.p. 60–70°) yielded the crude phosphorane (1·58 g), m.p. 180–210°. This product was dissolved in hot 2 N HCl (20 ml) and, on cooling, α-formylethyltriphenylphosphonium chloride (1·71 g), m.p. 220–225°, separated. This phosphonium salt was dissolved in warm water (20 ml) and made basic with Na_2CO_3, when the phosphorane (XXII) separated (1·34 g, 43 per cent), m.p. 218–221°, lit.[19] 220–222°, having a specific activity of $7·71 \times 10^7$ dpm/mM.

2,6-*Dimethyl-8,8-ethylenedioxy-2-octenal-2-^{14}C (XVIII)*

4-Methyl-6,6-ethylenedioxyhexanal (490 mg) and the phosphorane (XXII) (1·40 g, $6·9 - 10^7$ dpm/mM) were dissolved in benzene (11 ml) and the mixture refluxed for 24 hr in N_2. Evaporation of the benzene yielded an oily residue which was extracted with hot petroleum ether (b.p. 30–60°) (4 × 25 ml). The residue obtained on evaporation of this extract was chromatographed on silica gel. Evaporation of the fractions obtained by elution with a mixture of benzene and ether (9:1) yielded the aldehyde (XVIII), which was distilled, b.p. 70° (0·05 mm) affording a colourless oil (465 mg, 76 per cent). The product was 98 per cent pure by gas chromatography and was identical (i.r. spectrum, gas chromatography) with a sample of the aldehyde obtained by the oxidation of compound XVI with SeO_2.[20]

Iridodial-7-^{14}C

The previously described aldehyde (XVIII) (465 mg) was dissolved in 50 per cent aqueous formic acid (7 ml) and refluxed for 1 hr in N_2. The cooled reaction mixture was diluted with saturated brine (40 ml) and extracted with ether (2 × 20 ml). The ethereal extract was washed with water (2 × 15 ml), 2 N $NaHCO_3$ (2 × 15 ml), and more water (2 × 15 ml), before drying over $MgSO_4$. The oil obtained on evaporation of the ether was distilled *in vacuo* at 80° (0·005 mm) yielding iridodial-7-^{14}C as a pale-yellow oil (270 mg, 73 per cent) having a specific activity of $7·05 \times 10^7$ dpm/mM. The labelled product was identical with material obtained from the aldehyde (XVIII) by Robinson's method.[20] *Anal.* Calc. for $C_{10}H_{16}O_2$ (168): C, 71·46; H, 9·52. Found: C, 71·86; H, 9·42 per cent. The mass spectrum had a parent peak at *m/e* 168. It gave a *bis*-2,4-dinitrophenylhydrazone, m.p. 223–225°, lit.[32] 224–225°.

Administration of Iridodial-7-^{14}C to Vinca rosea *and Isolation of the Vindoline*

Iridodial-7-^{14}C (270 mg, $1·13 \times 10^8$ dpm) was emulsified in water (450 ml) with the aid of *Tween* 80 (10 drops). About 120 shoots were cut from 6-month-old *V. rosea* plants, and the cut ends were placed in the aqueous emulsion containing the iridodial. The plants were harvested after 16 days, when only 2 per cent of the original activity remained in the aqueous solution. The crude alkaloids (0·62 g) were isolated from the fresh shoots (461 g) as previously described.[1] The fractions containing vindoline were purified by TLC on alumina PF_{254} (Merck), eluting with ethyl acetate. Vindoline had an R_f of 0·3–0·4, and was extracted from this zone with a mixture of $CHCl_3$ and methanol. Evaporation yielded vindoline (41·6 mg). Recrystallization from ether yielded material having an activity of only 16 dpm/mg.

Acknowledgement—This investigation was supported by a research grant GM-13246 from the U.S. Public Health Service.

[31] Purchased from Volk Isotopes, Chicago, Illinois.

[32] G. W. K. Cavill, D. L. Ford and H. D. Locksley, *Australian J. Chem.* **9**, 288 (1956).