Phytochemistry, 1972, Vol. 11, pp. 2221 to 2231. Pergamon Press. Printed in England.

BIOSYNTHESIS OF DITERPENES IN *BEYERIA LESCHENAULTII*

H. J. BAKKER, E. L. GHISALBERTI and P. R. JEFFERIES

Department of Organic Chemistry, University of Western Australia, Nedlands 6009 Australia

(*Received* 4 *December* 1971, *in revised form* 1 *February* 1972)

Key Word Index—*Beyeria leschenaultii*; Euphorbiaceae; diterpene biosynthesis; beyerene; beyeren-19-ol; 3,4-secobeyerene acid.

Abstract—Beyerene and beyeren-19-ol have been incorporated into the diterpenoids of *Beyeria leschenaultii*. Both serve as precursors of beyerol, 17,19-dihydroxybeyer-15-en-3-one and the 3,4-secobeyerene acid (I) but only beyerene is incorporated into 6β,17-dihydroxybeyer-15-en-3-one, the major component. The significance of beyerene and beyeren-19-ol as precursors of (I) is discussed with reference to possible mechanisms for its formation.

INTRODUCTION

THE OBSERVED difficulty of incorporating mevalonic acid into the cyclic diterpenes of higher plants[1] has until recently restricted the investigation of their biosynthesis and interest has been focused[2] on the diterpenes from fungal organisms which are more amenable to this approach. A partial solution to this problem has been the preparation[3] of cell-free enzyme systems from a number of plants and these have been shown capable of incorporating mevalonic acid into di-, tri- and tetracyclic diterpene hydrocarbons.[3,4] This approach, however, is at present not applicable to most plants or to the study of the biosynthesis of polyoxygenated diterpenes.

The apparent reluctance of mevalonic acid to enter the diterpene cycle has been rationalized by invoking compartmentalization mechanisms[5] which do not allow exogenous mevalonic acid a ready entry to the site of biosynthesis.[6] Incorporation of precursors which need only undergo secondary transformations can bypass this obstacle and remains a more general approach to the study of the biosynthesis of many plant diterpenes. We have adopted this approach in an investigation of the biosynthesis of the seco-acid (I) which has previously

---

[1] H. J. NICHOLAS, in *Comprehensive Biochemistry* (edited by M. FLORKIN and E. H. STOTZ), Vol. 20, p. 1, Elsevier, Amsterdam (1968).

[2] J. R. HANSON and A. F. WHITE, *Chem. Commun.* 1071 (1969); R. EVANS, J. R. HANSON and A. F. WHITE, *J. Chem. Soc.* **C**, 2601 (1970) and references therein; B. ACHILLADELIS and J. R. HANSON, *J. Chem. Soc.* **C**, 2010 (1969); K. D. BARROW, D. H. R. BARTON, E. B. CHAIN, C. CONLAY, T. V. SMALE, R. THOMAS and E. S. WAIGHT, *Chem. Commun.* 1195 (1968); A. J. BIRCH, C. W. HOLZAPFEL and R. W. RICKARDS, *Tetrahedron Suppl.* **8**, Part II, 359 (1966); D. ARIGONI, *Pure Appl. Chem.* **17**, 331 (1968).

[3] J. E. GRAEBE, *Planta* **85**, 171 (1969); *Phytochem.* **7**, 2003 (1968); J. D. ANDERSON and T. C. MOORE, *Plant Physiol.* **42**, 1527 (1967); J. E. GRAEBE, D. T. DENNIS, C. D. UPPER and C. A. WEST, *J. Biol. Chem.* **240**, 1847 (1965).

[4] D. R. ROBINSON and C. A. WEST, *Biochem.* **9**, 70 (1970).

[5] T. W. GOODWIN, in *Rodd's Chemistry of Carbon Compounds* (edited by S. COFFEY), 2nd Edition, Vol. IIE, Chap. 19, p. 54, Elsevier, Amsterdam (1971).

[6] G. R. WALLER, in *Progress in the Chemistry of Fats and other Lipids* (edited by R. T. HOLMAN), Vol. X, Part 2, Pergamon Press, Oxford (1969).

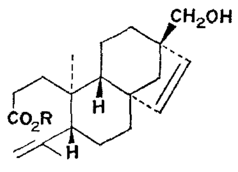
been reported[7] as a constituent of *Beyeria leschenaultii.* Although I represents the first of only two examples[8] of a diterpene with the A-seco ring structure, this feature is more commonly encountered in the triterpene series.[9]

This communication reports the synthesis and incorporation of $[17,19\text{-}^3H_2]$-beyerene, $[17\text{-}^3H_2]$-beyeren-19-ol, and $[17\text{-}^3H_2]$-beyerol into the diterpenes of *B. leschenaultii.* The results obtained are discussed with reference to the possible mechanisms of formation of the seco-acid (I).

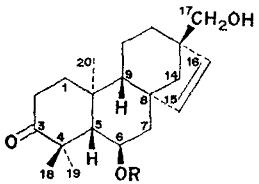
RESULTS

Isolation of the Diterpenes of B. leschenaultii

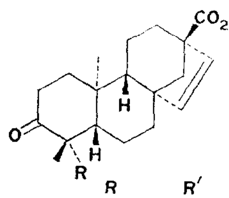
The occurrence of the seco-acid (I), keto acetoxy alcohol (III) and the keto acetoxy acid (V) in *Beyeria leschenaultii* has been reported elsewhere.[7] Preliminary experiments indicated that for the purpose of the biosynthetic studies reported here the isolation procedure could be simplified by directly saponifying the ether extract from the plant. The acidic fraction obtained, after esterification, afforded the seco-ester (II) and the keto ester (VI),[10] the latter arising from (V) via a retro-aldol reaction. The neutral fraction consisted mainly of the keto diol (IV)[7] but closer investigation led to the isolation of the nor-keto alcohol (VIII) and detection of trace amounts of beyerol (IX)[10] and beyeren-19-ol (X).[11] The nor-keto alcohol (VIII) was isolated by column chromatography of the neutral fraction. Physico–chemical properties were consistent with the structure assigned and confirmation of this was obtained by comparison with an authentic sample (see Experimental). The nor-compound probably arises from 17,19-dihydroxybeyer-15-en-3-one (VII), its corresponding mono- or diester derivatives, or from a 3-oxobeyeren-19-al precursor on saponification of the extract. Beyerol (IX), difficultly separable from the keto diol (IV), was isolated as its triacetate and identified



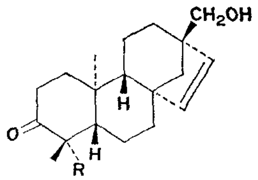
(I) R = H
(II) R = CH$_3$



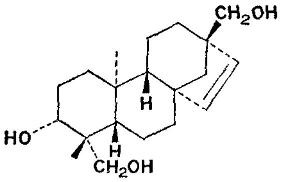
(III) R = Ac
(IV) R = H



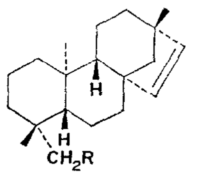
	R	R′
(V)	CH$_2$OAc	H
(VI)	H	CH$_3$



(VII) R = CH$_2$OH
(VIII) R = H



(IX)



(X) R = OH
(XI) R = H

---

[7] E. L. Ghisalberti and P. R. Jefferies, *Austral. J. Chem.* **21,** 439 (1968).

[8] P. W. Chow and P. R. Jefferies, *Austral. J. Chem.* **21,** 2529 (1968).

[9] R. McCrindle and K. H. Overton, in *Rodd's Chemistry of Carbon Compounds* (edited by S. Coffey), 2nd Edition, Vol. IIC, Chap. 14, p. 406, Elsevier, Amsterdam (1969).

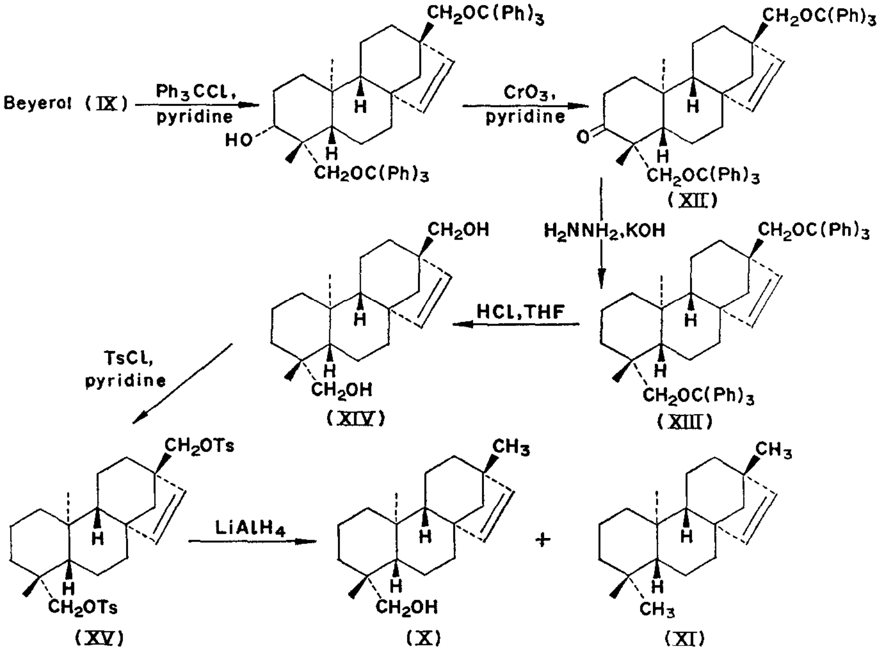
[10] P. R. Jefferies, R. S. Rosich, D. E. White and M. C. Woods, *Austral. J. Chem.* **15,** 521 (1962).

[11] R. D. H. Murray and R. McCrindle, *Chem. & Ind.* 500 (1964); R. McCrindle, A. Martin and R. D. H. Murray, *J. Chem. Soc.* C, 2349 (1968) and references therein.

by MS comparison with an authentic sample.[10] Beyeren-19-ol (X) was identified similarly by isolation of the appropriate fraction and MS comparison with an authentic sample.

Biosynthetic Studies

Our interest in the biosynthesis of diterpenoids was focused on the seco-acid (I) principally because the A-seco ring structure represents an interesting step in plant secondary metabolism and one which is being encountered more frequently in terpenoid natural products.[9,12,14,15] Furthermore, processes which result in bond fragmentation have recently received some attention in the biosynthesis of simpler terpenes[13,16] and have been implicated in the biosynthesis of other terpenoids.[12,17,18] The work reported here was an attempt to answer three major points concerning the biosynthesis of the seco-acid; (i) can the seco-acid be biosynthesized from the parent tetracyclic compound-beyerene (XI)? If so, (ii) is the ring opening reaction stereoselective for one of the C-4 methyl groups?; (iii) what is the level of oxidation required at C-3 and at either of the C-4 methyl groups? Our approach to these problems was to synthesize and test as precursors radioactively labelled beyerene, beyeren-19-ol, and beyerol.



Scheme 1.

---

[12] D. L. Dreyer, in *Progress in the Chemistry of Organic Natural Compounds* (edited by W. Zeichmeister), Vol. XXVI, p. 190, Springer–Verlag, Berlin (1968).

[13] A. R. Battersby, in *Natural Substances Formed Biologically from Mevalonic Acid* (edited by T. W. Goodwin), p. 157, Academic Press, New York (1970).

[14] K. Nakanishi and K. Habaguchi, *J. Am. Chem. Soc.* **93**, 3546 (1971).

[15] K. Habaguchi, M. Watanabe, Y. Nakadaira, K. Nakanishi, A. K. Kiang and F. Y. Lim, *Tetrahedron Letters* 3731 (1968); G. R. Chopra, A. C. Jain and T. R. Seshadri, *Indian J. Chem.* **7**, 1179 (1969); *ibid.* **8**, 401 (1970).

[16] C. J. Coscia, L. Botta and R. Guarnaccia, *Arch. Biochem. Biophys.* **136**, 498 (1970).

[17] M. Biollaz and D. Arigoni, *Chem. Commun.* 633 (1969); A. Corbella, P. Gariboldi, G. Jommi and C. Scolastico, *Chem. Commun.* 634 (1969).

[18] W. Parker, J. S. Roberts and R. Ramage, *Quart. Rev.* **21**, 331 (1967).

Synthesis of labelled precursors. Beyerene (XI) and beyeren-19-ol (X) could be prepared without complications from beyerol (IX) as shown in Scheme 1. For the preparation of tritium labelled (X) and (XI) lithium aluminium tritiide was used in the reduction of the ditosylate (XV). This reduction afforded [17,19-$^{3}H_{2}$]-beyerene and [17-$^{3}H_{1}$]-beyeren-19-ol. [17,19-$^{3}H_{2}$]-Beyerene was purified by preparative GLC and had specific activity of 3·24 × 10^{6} dpm/mg. [17-$^{3}H_{1}$]-Beyeren-19-ol was isolated and purified by chromatography and had specific activity 1·52 × 10^{6} dpm/mg. The choice of Scheme 1 as a synthetic route to (X) and (XI) was determined by the need to label selectively one of the C-4 methyl groups in beyerene which may be distinguishable in the product. As discussed below evidence was obtained that the C-19 of beyerene becomes the methylene carbon in the seco-acid and this provided a basis for the assumption that beyeren-19-ol (X) might also be a precursor of the seco-acid (I). Following the successful incorporation of (X) and (XI) tritium labelled beyerol was prepared by reduction of the dihydroxy ester (XVI)[10] with lithium aluminium tritiide. The [17-$^{3}H_{2}$]-beyerol (2·2 × 10^{6} dpm/mg) thus obtained was tested as a precursor of the seco-acid (I).

Incorporation studies. Labelled precursors were administered to the underside of the leaves of *Beyeria leschenaultii* seedlings over a period of 9–10 days. The results of the incorporation of (IX), (X) and (XI) and percentage incorporation into the diterpenoids of the plant are summarized in Table 1. Percentage incorporations were calculated as a ratio of the amount of precursor utilized. For (X) and (IX) two incorporation experiments were carried out at different periods of the year.

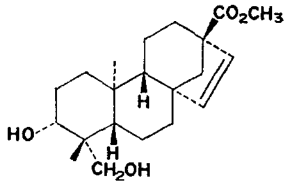
TABLE 1. INCORPORATION OF LABELLED PRECURSORS INTO THE DITERPENES OF *Beyeria leschenaultii*

Precursor	% recovered	% incorporation[*] Seco-acid[†] (I)	17,19-Dihydroxy beyeren-3-one[‡] (VII)	6β,17-Dihydroxy beyeren-3-one (IV)	Beyerol (IX)
[17,19-$^{3}H_{2}$]-beyerene (XI)					
(1) (February)	6·4	0·21	0·28[‖]	0·01	0·16
[17-$^{3}H_{1}$]-beyeren-19-ol (X)					
(2) (March)	24·9	0.26	0·26	—	—
(3) (December)	83·9	0·28	0·29	0·0[§]	0·1
[17-$^{3}H_{2}$]-beyerol (IX)					
(4) (December)	66·3	0·04	0·16	—	—
(5) (February)	56·1	< 0·01	0·55	—	—

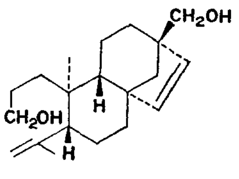
[*] Calculated on precursor utilized.
[†] From constant specific activity of seco-diol (XVII).
[‡] From constant specific activity of nor-ketone (VIII).
[§] All activity lost after two recrystallization of its diacetate.
[‖] Could not be recrystallized to constant specific activity.

In Experiment 1 purification of the keto diol (IV), achieved via its diacetate, indicated that the major portion of the radioactivity was associated with beyeryl triacetate. The nor-ketone (VIII) obtained from this experiment could not be recrystallized to constant specific activity. Since beyeren-19-ol (X) is difficultly separable from the nor-ketone (VIII) part of the radioactivity is believed to be associated with this compound. Evidence for this is indicated from experiments 4 and 5, where little difficulty was experienced in obtaining the nor-ketone radioactively pure (beyeren-19-ol would not be expected to incorporate label from beyerol).

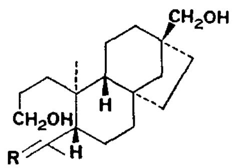
The acidic fraction of the extract from Experiment 1 after esterification was easily separable into two fractions corresponding to the nor-keto ester (VI) and the seco-ester (II). The former, as expected, lost all radioactivity after one recrystallization. The seco-ester was purified by preparative TLC and then reduced to the seco-diol (XVII) which was recrystallized to constant specific activity. The radioactive sample of seco-diol (XVII) was diluted with carrier material and this fraction (2·24 × 10^5 dpm/mmol) was utilized for degradation purposes. Previous work[7] on the structural elucidation of the seco-acid (I) had revealed that the cyclopentene double bond could be selectively hydrogenated in the presence of the methylene group. The isolation of the methylene carbon in the resultant dihydroseco-diol (XVIII) could then be simply realized. In fact hydrogenation of (XVII) with Pd–C in EtOH gave the dihydro diol (XVIII) which was purified by conversion to the tetrol (XIX) with osmium tetroxide. The specific activity of the tetrol (1·08 × 10^5 dpm/mmol) showed that 50% of the radioactivity had been lost during hydrogenation. This was confirmed by oxidation of the tetrol (XIX) with $NaIO_4$ to the nor-ketone (XX) which was characterized as the oxime (XXI). The latter showed similar specific activity (1·05 × 10^5 dpm/mmol) to that of the tetrol (XIX).



(XVI)



(XVII)



(XVIII) R = CH_2
(XIX) R = $<^{OH}_{CH_2OH}$
(XX) R = O
(XXI) R = NOH

The exchange of olefinic protons by hydrogen catalysts is well recognised and this result indicated that the methylene protons of (XVII) were tritium labelled. That the allylic protons in (XVII) were not exchanged cannot be excluded although this process would not be expected to lead to complete exchange under the hydrogenation conditions used.[19] Although not conclusive, this result indicated that the ring opening reaction was stereoselective for the C-4 axial methyl of beyerene.

To confirm this, labelled beyeren-19-ol (X) was tried as a precursor (Experiments 2 and 3). The seco-acid recovered from these experiments showed significant incorporation of label as did the nor-ketone (VIII) and beyerol. To investigate further the requirements for C–C bond fragmentation, labelled beyerol was the next substrate utilized (Experiments 4 and 5). In this case, however, only the nor-ketone (VIII) was significantly labelled whereas the seco-acid (I) showed minimal incorporation.

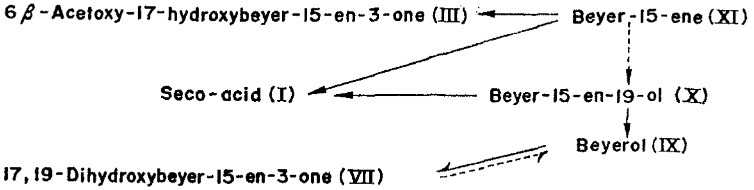
DISCUSSION

The results presented in Table 1 clearly indicate that exogenous precursors can enter into the diterpenoid biosynthetic pathway of *Beyeria leschenaultii*. The percentage incorporation of beyerene is not significantly different from that of the other two precursors although it is more efficiently utilized. That seasonal differences may be important in determining

---

See for example: E. Caspi and L. J. Mulheirn, *J. Am. Chem. Soc.* **92**, 404 (1970).

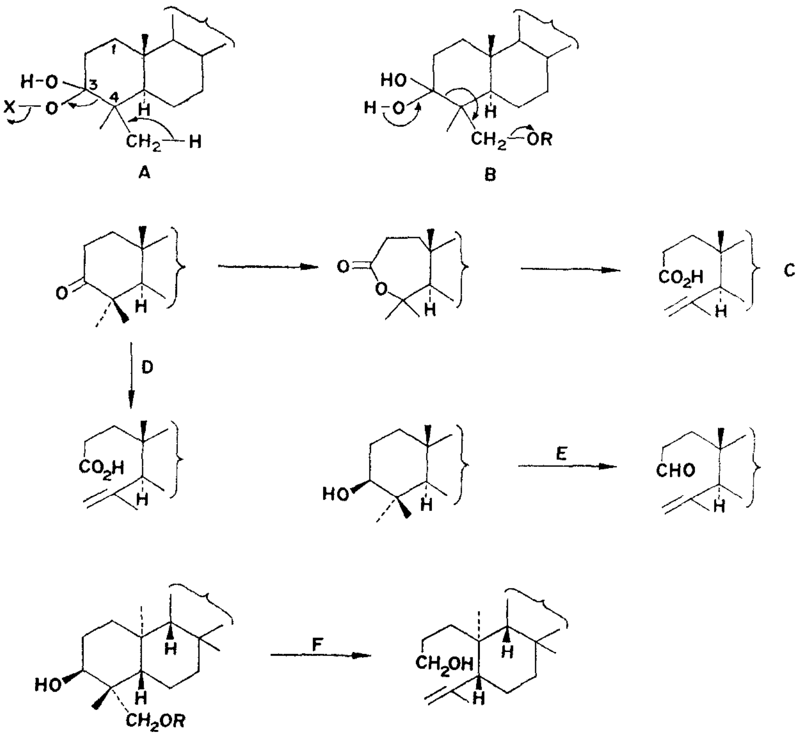
efficiency of incorporation is evident from Experiments 2 and 3. Furthermore for the more polar beyeren-19-ol and beyerol penetration of the leaf surface of the plant must be relatively more difficult. The results in Table 1 can be summarized in diagrammatic form in Scheme 2. The sequence represented here is meant to indicate possible steps in the biosynthesis of the diterpenes in *Beyeria leschenaultii* and does not necessarily imply obligatory intermediates or pathways.



Scheme 2.

It is of some significance that both beyeren-19-ol and beyerol have been detected as constituents of this plant. Attempts to detect beyerene in the extracts were hindered by the presence of large quantities of fatty material.

The results reported here provide some evidence for the stereochemistry of the seco-A-ring formation in the biosynthesis of (I). That the C-19 methyl group in beyerene becomes the methylene group in the seco-acid finds confirmation in the observed incorporation of beyeren-19-ol into this acid. Formal schemes (A, B and C) have been suggested[20–22] to



---

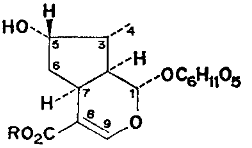
[20] D. Arigoni, D. H. R. Barton, R. Bernasconi, C. Djerassi, J. S. Mills and R. E. Wolff, *J. Chem. Soc.* 1900 (1960).

[21] G. H. Whitham, *J. Chem. Soc.* 2016 (1960).

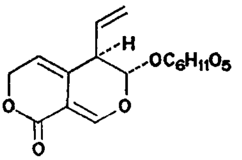
[22] A. I. Laskin, P. Grabwich, C. Meyers and J. Fried, *J. Med. Chem.* **7**, 406 (1964).

explain the formation of the A-seco ring feature observed for some triterpenes. Furthermore this feature can be generated chemically in a number of ways from suitable precursors. Of these, three are relevant here. Photolysis of the 3-oxo-4,4-dimethyl system in the presence of oxygen affords the unsaturated seco-acid (Scheme D).[23] Lead tetracetate oxidation of a 3-hydroxy-4,4-dimethyl system leads to the seco-aldehyde (Scheme E).[24] The corresponding seco-alcohol can be generated by $LiAlH_4$ treatment of the monoester (Scheme F).[7]

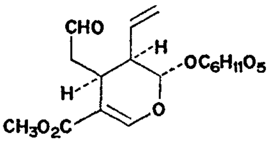
The stereoselectivity observed in the formation of the diterpene seco-acid (I) is also implied in the mechanisms of type A, B (where the C-4 axial group is involved), and F, but would not be expected for the processes D and E where radicals are involved. That mechanisms D or E were operating non-enzymatically could not *a priori* be excluded. Mechanism C has been shown[25] to proceed chemically without selectivity for the methyl groups but the same cannot be supposed for an enzyme controlled pathway. However, the incorporation of beyeren-19-ol (X) to the seco-acid (I) indicates that, for this pathway at least, mechanisms of type A, C, D, E can be excluded and argues well for B or F. Although both beyerene (XI) and beyeren-19-ol (X) could be on different pathways to the seco-acid (I), it seems reasonable to assume that the sequence beyerene to a 19-hydroxylated beyerene to seco-acid is operating. A choice between mechanism B and F requires either a knowledge of the level of oxidation at C-3 and/or identification of the first formed seco compound.



(XXII) $R = H$
(XXIV) $R = CH_3$



(XXIII)



(XXV)

Evidence for these points is difficult to obtain since oxido-reductase activity is likely in these systems as indicated by the significant incorporation of beyerol (IX) into the dihydroxy ketone (VII) (Table 1). Some evidence relating to the mechanism and stereochemistry of one C–C bond fragmentation process is available from the study of the biosynthesis of the secoiridoids. Formation of gentiopicroside (XXIII) from its precursor loganic acid (XXII) proceeds[16] with retention of the C-5 proton. Loganin (XXIV) is incorporated into all three families of indole alkaloids via secologanin (XXV) with retention of the C-5 proton in the precursor.[13,26,27] The intermediacy of a 1,3-diol which could undergo fragmentation, after conversion of the primary hydroxyl into a suitable leaving group has been postulated[27] for the biosynthesis of secologanin (XXV). That a similar process is not operating in the biosynthesis of the seco-acid is indicated from the experiments in which beyerol was used as substrate. In these experiments only the nor-ketone (VIII) was significantly labelled and the poor incorporation into the seco-acid militates against beyerol and the dihydroxy ketone (VII) as true intermediates in the pathway to the seco-acid.

---

[23] G. Quinkert, *Angew Chem.* 4, 211 (1965).

[24] M. Amorosa, L. Caglioti, G. Cainelli, H. Immer, J. Keller, H. Wehrli, M. Lj. Mihailovic, K. Schaffner, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **45**, 2674 (1962).

[25] D. Rosenthal, *J. Org. Chem.* 32, 4084 (1967).

[26] A. R. Battersby, A. R. Burnett and P. G. Parsons, *J. Chem. Soc.* C, 1187 (1969).

[27] A. R. Battersby and K. H. Gibson, *Chem. Commun.* 902 (1971).

EXPERIMENTAL

General. Mp.s were determined on a Koffler block and are uncorrected. Microanalyses were carried out by the Australian Microanalytical Service. NMR spectra were recorded on a Varian A60 Spectrometer for $CHCl_3$ or $CDCl_3$ solutions, unless otherwise stated, with tetramethylsilane as an internal standard. IR spectra were recorded in CS_2. Rotations were determined for $CHCl_3$ solutions in 1-d.m. tubes at room temp. Preparative GLC was carried out with an Aerograph A-700 Autoprep chromatograph (Column: 8′, 15% DEGS on Chromosorb W, carrier gas: Helium at 10 psi). For preparative and analytical TLC silica gel plates (0·25 mm) were used. Unless otherwise stated plates were developed in $CHCl_3$–MeOH (47:3). Radioactivity measurements were made using a Nuclear Chicago 720 Liquid Scintillation counter. The samples were dissolved in 10 ml of a scintillator solution containing 2,5-diphenyloxazole (0·7 g), 2-*p*-phenylenebis(5-phenyloxazole) (0·05 g), naphthalene (5 g) in dioxan (100 ml).

Isolation of minor constituents. 17-*Hydroxy*-19-*norbeyer*-15-*en*-3-*one* (VIII). The ether soluble extract of the plant was saponified with aq. NaOH (10%) and the neutral fraction recovered was adsorbed on a column of Neutral Alumina (Act II). Elution with benzene–Et_2O (9:1) gave a small amount of the *nor-ketone* (*VIII*). Further elution with benzene–Et_2O (1:1) afforded the keto diol (*IV*). The nor-ketone recrystallized from light petroleum as plates, m.p. 119–120°, $[\alpha]_D$ +20° (*c*, 0·3) (Found: C, 79·11; H, 9·94. $C_{19}H_{28}O_2$ requires: C, 79·12; H, 9·79%). The NMR spectrum showed signals at 0·85δ (3H, C-10 methyl), 1·00δ (3H doublet; *J* = 7 Hz; C-19 methyl). 3·52δ (2H singlet; $-C\underline{H}_2-OH$) and at 5·66 and 5·90δ (2H quartet; *J* = 6 Hz; vinyl protons). An authentic sample of the nor-ketone was obtained by acid hydrolysis of the ditrityl ketone (*XII*). Comparison of the two samples (m.p., m.m.p., TLC, NMR, and MS) showed them to be identical. From the hydrolysis a small amount of 17,19-*dihydroxybeyer*-15-*en*-3-*one* (VII) was obtained. This compound recrystallized from acetone as prisms, m.p. 177–179°, $[\alpha]_D$ +23° (*c*, 0·8), M^+ 318, (Found: C, 75·65; H, 9·46, $C_{20}H_{30}O_3$ requires: C, 75·43; H, 9·50%). The NMR spectrum (C_5H_5N) showed signals at δ, 1·04, 1·43 (3H singlets, tertiary methyls), 3·75 (2H singlet; C-17 methylene protons), 3·79 and 4·33 (AB quartet; *J* = 11 Hz; C-19-methylene protons) and 5·79, 5·90 (2H singlets; $O\underline{H}$ and vinylic protons.)

Beyer-15-*en*-19-*ol* (X). In another experiment preparative TLC of the neutral fraction and separation of the band whose R_f corresponded to that of authentic beyer-15-en-19-ol gave a fraction whose NMR spectrum indicated it to be mainly fatty material. MS analysis of this fraction (scans were recorded at different temps) showed that beyer-15-en-19-ol was present (comparison with MS of an authentic sample).

Beyerol (IX). Since beyerol and the keto-diol (IV) showed similar chromatographic properties (see Table 2) separation of the two was achieved as follows: the keto-diol fraction was acetylated with Ac_2O–pyridine and the product obtained reduced with $NaBH_4$. Preparative TLC of this fraction and isolation of a band whose R_f corresponded to that of beyeryl triacetate gave a compound whose MS resulted identical to that of an authentic sample of beyeryl triacetate.[10] Strict precautions were taken so that fractions were not contaminated by authentic materials used as markers (Table 2).

TABLE 2. R_f* VALUES OF SOME HYDROXYLATED BEYERENES

17-Hydroxy-19-norbeyer-15-en-3-one (VIII)	0·56
17,19-Dihydroxybeyer-15-ene (XIV)	0·45
3,17-Dihydroxybeyer-15-ene	0·45
17,19-Dihydroxybeyer-15-en-3-one (VII)	0·37
6,17-Dihydroxybeyer-15-en-3-one (IV)	0·30
Beyerol (IX)	0·30
VII + IX	0·45; 0·32
VII + IV	0·45; 0·34

* Solvent system–$CHCl_3$:6% MeOH

Synthesis of labelled precursors. Beyerol (IX:6·0 g), Ph_3CCl (12·0 g) in dry benzene (130 ml) and pyridine (10 ml) were heated under reflux for 48 hr. The cooled solution was filtered, the solvent evaporated and the residue dissolved in ether, washed with water and dried. Evaporation of the ether and recrystallization from acetone afforded 3α-*hydroxy*-17,19-*ditritoxybeyer*-15-*ene* (13·8 g), m.p. 168–170°. ν_{max}:3480 (OH), 3050, 745 (disubstituted double bond), 3020, 755 and 705 cm^{-1} (aromatic rings). NMR resonances at δ0·18, 1·53 (3H singlets; tertiary methyls), 2·90 (2H singlet; C-17 protons), 3·38 and 3·13 (AB quartet; *J* = 9 Hz; C-19 protons), 5·54 (2H singlet; vinylic protons) and 7·37 (30H multiplet; aromatic protons). The ditritoxybeyerol (10·0 g) in pyridine (100 ml) was treated with CrO_3 (3·4 g) in pyridine (34 ml) overnight. The product recovered with ether was recrystallized from acetone-light petroleum as prisms of 3-*oxo*-17,19-*ditritoxybeyer*-15-*ene* (XII; 8·28 g), m.p. 233–234°, $[\alpha]_D$ +35° (*c*, 1·4) (Found: C, 85·98; H, 7·44. $C_{58}H_{58}O_3$. $[(CH_3)_2CO]_{\frac{1}{2}}$ requires C, 85·92; H, 7·34%). The NMR spectrum of XII showed a resonance at 2·03δ assigned to acetone protons. The integral showed that acetone solvates the ketone in a ratio of 0·5:1. Other resonances were observed at δ0·48, 1·30 (3H singlets; tertiary methyls), 2·88 (2H singlet; C-17 protons), 3·41, 3·03 (AB quartet; C-19 protons), 5·57 (2H singlet; vinylic protons) and at 7·33 (aromatic protons). ν_{max} 1720 cm^{-1} (cyclohexanone). The ketone (XII; 8·0 g) in dioxan (100 ml), diethylene glycol (230 ml) and hydrazine hydrate (30 ml) were heated under reflux in N_2 for 2·5 hr at 195°. The product recovered with ether was filtered through alumina with light petroleum. The fraction obtained was recrystallized from the same solvent to give cubes of 17,19-*ditritoxybeyer*-15-*ene* (XIII; 6·77 g) m.p. 224–225°, $[\alpha]_D$ +12° (*c*, 1·4). (Found: C, 88·18; H, 7·94. $C_{58}H_{60}O_2$ requires: C, 88·33; H, 7·62%.) The ditritoxybeyerene (XIII; 4·52 g) in the THF (200 ml) and aq. HCl (25%; 40 ml) was heated under reflux for 1·5 hr. The product recovered was adsorbed on Neutral Alumina (Act III). Elution with $CHCl_3$–light petroleum (1:4) gave triphenylcarbinol (2·07 g). Further elution with $CHCl_3$–light petroleum (1:1) gave a fraction which crystallized from the same solvent as cubes of beyer-15-ene-17,19-diol (XIV; 1·68 g), m.p. 85–187°, $[\alpha]_D$ +58° (*c*, 1·28), (lit.,[11] 179–181°, $[\alpha]_D$ +60°). The NMR spectrum of (XIV) was identical with that quoted.[11]

The diol (XIV; 670 mg) in pyridine (33 ml) was treated with toluene-*p*-sulphonyl chloride (532 mg) at 0° for 18 hr. The product recovered was adsorbed on Neutral Alumina (Act III; 25 g) from $CHCl_3$–light petroleum. Elution with 15% $CHCl_3$–light petroleum gave the ditosylate (15; 271 mg). Elution with 25% $CHCl_3$–light petroleum afforded 19-*hydroxybeyer*-15-*en*-17-*yl toluene*-p-*sulphonate* (352 mg) which recrystallized from Et_2O–MeOH as needles, m.p. 156–158° $[\alpha]_D$ +59° (*c*, 0·2). (Found C, 70·35; H, 8·42; S, 6·80. $C_{27}H_{38}O_4S$ requires: C, 70·68, H, 8·37; S, 6·99%.) Unreacted diol (150 mg) was recovered by elution with $CHCl_3$. The ditosylate was obtained more conveniently by treating the diol (XIV) with excess toluene-*p*-sulphonyl chloride under the same condition. The ditosylate (XV) recrystallized from EtOH as needles, m.p. 159–160°, $[\alpha]_D$ +34° (*c*, 2·2). (Found: C, 66·65; H, 7·24; S, 10·44. Calc. for $C_{34}H_{44}O_6S_2$: C, 66·28; H, 6·96; S, 10·30%.) (lit.,[11] 158–160°).

The ditosylate (XV; 1·02 g) and $LiAlH_4$ (326 mg) in dioxan (40 ml) was heated under reflux for 24 hr. The product recovered was adsorbed on a column of neutral alumina. Elution with light petroleum gave a hydrocarbon fraction (389 mg). This was purified by preparative vpc affording beyer-15-ene (XI; 99 mg), m.p. 28·5–30·5°, (lit.,[11] 30–33). Elution with $CHCl_3$–light petroleum (1:4) afforded beyer-15-en-19-ol (X; 94 mg) which recrystallized from light petroleum as needles, m.p. 119–120°, $[\alpha]_D$ +41 (lit.,[11] 119–120° $[\alpha]_D$ +39°). Spectroscopic properties of (X) and (XI) were identical with those quoted.[11] Beyer-15-en-19-ol could be obtained in better yields by similar reduction of the monotosylate.

[17,19-$^3H_2$]-*beyer*-15-*ene and* [17-$^3H_1$]-*beyeren*-19-*ol*. The ditosylate (XV; 500 mg) in dioxan (20 ml) was treated as above with $LiAlH_4$ (160 mg) and tritiated $LiAlH_4$ (1·9 mg; 5 mC). The product isolated was separated as described above yielding [17,19-$^3H_2$]-beyer-15-ene (63·4 mg; 3·24 × 10^6 dpm/mg) and [17-$^3H_1$]-beyer-15-en-19-ol (55 mg; 1·52 × 10^6 dpm/mg).

[17-$^3H_2$]-*beyerol*. The dihydroxy-ester (XVI; 70 mg) in tetrahydrofuran (5 ml) was reduced with $LiAlH_4$ (20 mg) and tritiated $LiAlH_4$ (1·25 mg; 2·5 mC). The product recovered (59·5 mg) was recrystallized from methanol as needles of [17-$^3H_2$]-beyerol (constant specific activity 2·2 × 10^6 dpm/mg).

Techniques of feeding and isolation of constituents. The radioactive precursors in acetone were applied as microdrops to the under-side of the leaves at the upper third portion of newly emerged stems of *Beyeria leschenaultii* daily for 5 days. After each application the leaves were sprayed with a silicone oil solution (10% in light petroleum). The seedlings were processed 5 days after the last application. The stem and leaves were ground, extracted with ether and ethanol and the crude extract in ethanol was saponified by refluxing with aq. NaOH (5%) for 1–2 hr. The solution was separated into a neutral and an acid fraction. Preliminary separation of the neutral constituents was achieved by column chromatography. Preparative TLC of the appropriate fraction was then used to purify individual components. In experiments 1–3 three fractions were taken corresponding to, (a) unutilized precursor, (b) nor-ketone (VIII), and (c) keto diol (IV). In experiments 4 and 5 no attempt was made to separate the keto diol from beyerol.

The acidic fraction was methylated with diazomethane or CH_3I–K_2CO_3 and the seco-ester (II) separated and purified by repeated preparative TLC. In experiment 1 (Table 3) the nor-keto ester (VI) was also isolated and identified. On recrystallization the nor-keto ester lost all radioactivity.

Purification of 6,17-*dihydroxybeyer*-15-*en*-3-*one* (IV). The radioactive fraction (30 mg) was acetylated with Ac_2O–pyridine and the diacetate purified by preparative TLC. The material recovered (20·5 mg) was recrystallized from light petroleum to constant specific activity (39 040 dpm/mmol). The MS of the diacetate (M^+ 402) showed no peak corresponding to beyeryl triacetate (MW 446). However closer investigation of this fraction in later experiments led to the isolation of trace amounts of beyeryl triacetate. The significant loss in radioactivity after the first two recrystallizations of the diacetate can be attributed, in part, to the presence of radioactively labelled beyeryl triacetate.

Purification and degradation of the seco-ester (II). The radioactive seco-ester (II; 17 mg) was treated with $LiAlH_4$ in boiling tetrahydrofuran for 3 hr. Recovery of the product in the normal way gave the seco-diol (XVII; M^+ 304) which was diluted with carrier material (43 mg) and recrystallized from benzene–petrol to constant specific activity (2·24 × 10^5 dpm/mmol). The radioactive seco-diol (XVII; 43 mg) in EtOH was hydrogenated over Pd–C (10%) for 1 hr. The product recovered was recrystallized from light petroleum–ether as needles of the dihydroseco-diol (XVIII; M^+ 306; m.p. 120–122°) (lit.,[7] 123–124°). The latter was

Table 3. Experiment 1: incorporation of [17,19-$^3H_2$]-beyer-15-ene

	Wt	Radioactivity (dpm)
[17,19-$^3H_2$]-beyer-15-ene	13·6 mg	22 × 10^6
Plant	4·0 g	—
Crude extract	2·1 g	—
Neutral fraction		
(a) Recovered beyerene	—	1·4 × 10^6
(b) Keto diol (IV)	30 mg	3·5 × 10^4
(c) nor-Ketone (VIII)	0·5 mg	5·8 × 10^4
Acidic fraction		
Seco-ester (II)	25 mg	7·2 × 10^4

treated with OsO_4–pyridine for 2 days at room temp. The product isolated (28·8 mg) was recrystallized from $CHCl_3$–light petroleum as prisms of the tetrol (XIX) (constant specific activity: 1·08 × 10^5 dpm/mmol). A sample of tetrol (XIX) prepared in a similar way from unlabelled dihydro seco-diol (XVIII) had the following characteristics: M^+ 340; NMR: signals at δ 1·13, 1·37 (3H singlets; tertiary methyls), 3·43 (2H singlets; C-19 protons), 3·60 (2H multiplet, C-3 protons) and 3·77 (2H, obscured signal, C-18 protons). The radioactive tetrol (18·0 mg) in dioxan–water (3:1) was treated with excess $NaIO_4$. The nor-keto diol (XX) recovered (17·9 mg; M^+ 308) was homogeneous on TLC but could not be crystallized. An authentic sample showed signals in its NMR spectrum at δ1·00 (3H singlet; C-10 methyl), 2·12 (3H singlet; C-19 protons), 3·42 (2H singlet; C-17 protons) and 3·63 (2H multiplet; C-3 protons). The radioactive nor-keto diol (XX) was diluted with carrier material (9·6 times) and treated with $NH_2OH{\cdot}HCl$ in pyridine. The oxime obtained was purified by preparative TLC and recrystallized from $CHCl_3$–ether as prisms (corrected specific activity. 1·05 × 10^5 dpm/mmol). An authentic sample prepared for comparison had the following characteristics, m.p. 162–163°, M^+ 323. (Found: 3·89. $C_{19}H_{33}O_3N$ requires: N, 4·33%). $[\alpha]_D$ −35° (*c*, 0·1). NMR (pyridine): 1·02, 2·03 δ (3H singlets; C_{10} and C-4 methyls respectively), 3·66 δ (2H broad singlet; C-17 hydrogens) and 3·87 δ (2H broad triplet; C-3 hydrogens).

Table 4. Experiments 2 and 3: incorporation of [17-$^3H_1$]-beyer-15-en-19-ol

	Exp. 2 (March)		Exp. 3 (December)	
	Wt	Radioactivity	Wt	Radioactivity
[17-$^3H_1$]-beyer-15-en-19-ol	13·3 mg	8·34 × 10^6	14·0 mg	8·83 × 10^6
Plant	5·0 g	—	2·8 g	—
Crude extract	—	—	800 mg	—
Neutral fraction	355 g	—	490 mg	—
(a) Beyer-15-en-19-ol (X)	8·8 mg	2·09 × 10^6	—	7·36 × 10^6
(b) Keto diol (IV)	—	negligible*	118 mg	negligible*
(c) nor-Ketone (VIII)	—	1·6 × 10^4	7·1 mg	1·7 × 10^4
Acidic fraction	—	—	243 mg	—
seco-Ester (II)	25·5 mg	4·3 × 10^4	63·5 mg	1·1 × 10^4

* After recrystallization of the diacetate.

Purification of constituents (see Table 4). *Experiment* 2. (i) The fraction corresponding to nor-ketone (VIII) was diluted with carrier material and recrystallized to constant specific activity: 526 dpm/mg. (% incorporation: 0·26). (ii) The fraction corresponding to the seco-ester (II) was purified by preparative TLC. The MS of the sample was identical to the authentic spectrum. The radioactive seco-ester (II; 23 mg) was reduced with $LiAlH_4$–THF and the product recovered was diluted with carrier material (27·1 mg) and recrystallized from benzene–light petroleum to constant specific activity: 326 dpm/mg. The radioactive seco-diol (XVII; 10·7 mg) in EtOH was hydrogenated over Pd–C for 2 hr at room temp. The product recovered was purified by preparative TLC and recrystallized from light petroleum–ether. The dihydroseco-diol (XVIII) had m.p. 122–124° (lit.,[7] 123–124°) and constant specific activity (397 dpm/mg).

Experiment 3. (i) The radioactive nor-ketone (VIII; 7·1 mg) was diluted with carrier material (23·3 mg) and recrystallized from acetone–light petroleum to constant specific activity (529 dpm/mg). (ii) The keto diol (IV) isolated was acetylated with Ac_2O–pyridine and the diacetate recrystallized from light petroleum. Two recrystallization gave non-radioactive diacetate. (iii) The radioactive seco-ester (II; 63·5 mg) was reduced to the seco-diol (XVI; 52·5 mg) which was recrystallized to constant specific activity (79 dpm/mg).

TABLE 5. EXPERIMENTS 4 AND 5: INCORPORATION OF [17-$^3H_2$]-BEYEROL

	Exp. 4 (December)		Exp. 5 (February)	
	Wt	Radioactivity	Wt	Radioactivity
[17-$^3H_2$]-Beyerol	13·5 mg	$2{\cdot}97 \times 10^7$	11·1 mg	$2{\cdot}44 \times 10^7$
Plant	3·5 g	—	2·75 g	—
Crude extract	0·9 g	—	—	—
Neutral fraction	398 mg	—	370 mg	—
(a) nor-Ketone (VIII)	2·8 mg	$1{\cdot}62 \times 10^4$	6 mg	$6{\cdot}2 \times 10^4$
(b) Beyerol (IX)	—	$1{\cdot}97 \times 10^7$	—	$1{\cdot}37 \times 10^7$
(c) Keto diol (IV)				
Acidic fraction	191 mg	—	250 mg	—
seco-Ester (II)	68·6 mg	$3{\cdot}7 \times 10^3$	37·1 mg	$1{\cdot}72 \times 10^4$

Purification of constituents (see Table 5). *Experiment* 4. (i) The radioactive nor-ketone (VIII; 28 mg) was diluted with carrier material (24·1 mg) and recrystallized to constant specific activity (605 dpm/mg). The nor-ketone was reduced with $NaBH_4$–MeOH and the compound obtained was separated by TLC (5% MeOH–$CHCl_3$) to give 3,17-dihydroxy-19-norbeyer-15-ene[12] (6·0 mg) which crystallized from acetone–light petroleum to constant specific activity 510 dpm/mg. (ii) The seco-ester (II; 68·6 mg) was reduced to the seco-diol (XVII). This was purified by preparative TLC and recrystallized to constant specific activity (81 dpm/mg).

Experiment 5. (i) The radioactive nor-ketone (VIII; 6·0 mg) was diluted with carrier material (13·5 mg) and recrystallized to constant specific activity ($3{\cdot}09 \times 10^3$ dpm/mg). (ii) The radioactive seco-ester (37·1 mg) was reduced to the seco-diol. This was purified by preparative TLC and the seco-diol (17·5 mg) recovered was diluted with carrier material (17·5 mg) and recrystallized. Constant specific activity could not be reached after four recrystallizations.