Phytochemistry, 1972, Vol. 11, pp. 3235 to 3245. Pergamon Press. Printed in England.

BIOSYNTHESIS OF PETASIN IN *PETASITES HYBRIDUS*

C. J. W. BROOKS and R. A. B. KEATES*

Chemistry Department, University of Glasgow, Glasgow G12 8QQ, Scotland

(*Received* 21 *December* 1971. *Accepted* 10 *May* 1972)

Key Word Index—*Petasites hybridus*: Compositae; biosynthesis; petasin; eremophilane.

Abstract—*Petasites hybridus* incorporates mevalonic acid into sesquiterpenoid esters of the petasin class. Base hydrolysis gave isopetasol containing 0·005% of the radioactivity administered to leaves, and 0·018% in the case of flowers. Degradations of the labelled isopetasol have demonstrated specific incorporation of the precursor in a manner consistent with the postulated mechanism.

INTRODUCTION

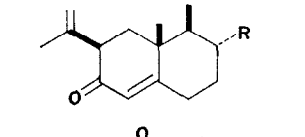
THE PETASIN esters (I—III), isolated from *Petasites hybridus* L.[1–3] are among the many examples of eremophilane (IV) type sesquiterpenoids found in *Petasites* and closely related genera of the Compositae.[4,5] The carbon skeleton of eremophilane is the classical example of a sesquiterpenoid structure that cannot be derived by direct cyclisation of farnesyl pyrophosphate, as formulated in Ruzicka's original hypothesis for sesquiterpenoid biosynthesis.[6,7] In the course of the biosynthesis of the eremophilane sesquiterpenoids, the methyl group at C-5 is presumed to arise by migration from C-10 in a precursor of eudesmanoid skeletal type (V), as originally proposed by Robinson.[8] We have now completed radioactive tracer experiments (using specifically labelled mevalonic acid) the results of which are consistent with the postulated mode of biosynthesis of petasin from *trans,trans*-farnesyl pyrophosphate. (Preliminary experiments on the incorporation of mevalonate into sesquiterpenoids of *P. hybridus* were carried out in collaboration with Zabkiewicz.[9])

It is reasonable to postulate the intermediacy of β-germacrene in the biosynthesis of eremophilane sesquiterpenoids. In accord with this is the observation that many of the sesquiterpenoids found in plants of the Compositae possess cyclic structures and stereochemistry that are readily derived from *chair,chair* folded β-germacrene having both ring methyl groups in an axial orientation.[4,7] Transannular reaction of such a *chair* folded precursor may be initiated by equatorial proton attack at C-1, giving rise to a eudesmanoid

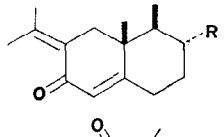
* Present address: MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, MI 48823, U.S.A.

[1] A. AEBI, J. BÜCHI, T. WAALER, E. EICHENBERGER and J. SCHMUTZ, *Pharm. Acta Helv.*, **29**, 277 (1955).
[2] A. AEBI and T. WAALER, *Uber die Inhaltsstoffe von Petasites hybridus (L) Fl. Wett.*, Helbing & Lichtenhahn, Basel (1959).
[3] A. AEBI and C. DJERASSI, *Helv. Chim. Acta*, **42**, 1785 (1959).
[4] V. HEROUT and F. ŠORM, in *Perspectives in Phytochemistry* (edited by J. B. HARBORNE and T. SWAIN), p. 139, Academic Press, London (1969).
[5] L. NOVOTNÝ, J. TOMAN, F. STARY, A. D. MARQUEZ, V. HEROUT and F. ŠORM, *Phytochem.* **5**, 1281 (1966).
[6] L. RUZICKA, *Experientia* **9**, 357 (1953); *idem. Proc. Chem. Soc.* 341 (1959).
[7] J. B. HENDRICKSON, *Tetrahedron* **7**, 82 (1959).
[8] R. ROBINSON, cited by A. R. PENFOLD and J. L. SIMONSEN, *J. Chem. Soc.* 87 (1939).
[9] J. A. ZABKIEWICZ, R. A. B. KEATES and C. J. W. BROOKS, *Phytochem.* **8**, 2087 (1969).

intermediate as a carbonium ion (Scheme 1). If petasin stereochemistry is to be attained, folding of the precursor on the enzyme surface must locate the methyl groups on the β-face, and the incoming proton becomes the 1β-(equatorial) substituent. When ring *A* is in the chair conformation, the hydrogen at C-5α and the angular methyl group at C-10β are both axial. With the carbonium ion protected from solvolysis by a hydrophobic environment, as may occur in the interior of an enzyme, neutralisation of the charge would follow by

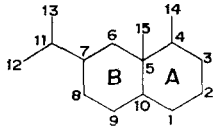


(I), R = —O—C(=O)—CH=C(CH$_3$)$_2$; Petasin
= —OAng (Angelate)

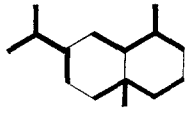


(II), R = —O—C(=O)—C(CH$_3$)=CH$_2$; Isopetasin

(III), R = —O—C(=O)—CH=C(CH$_3$)—S—CH$_3$; *S*-Isopetasin

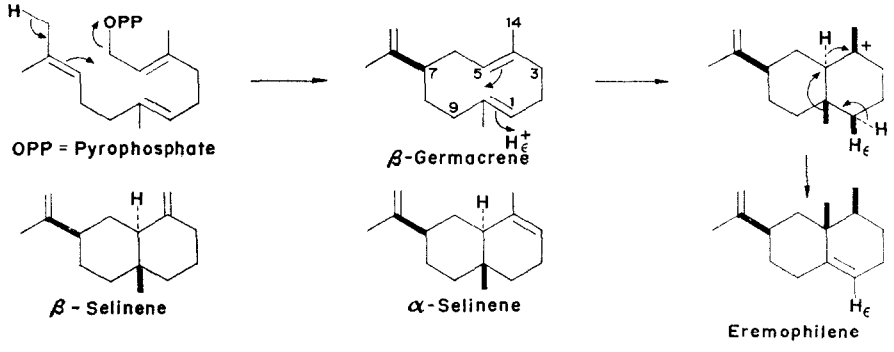


(IV), Eremophilane



(V), Eudesmane skeleton

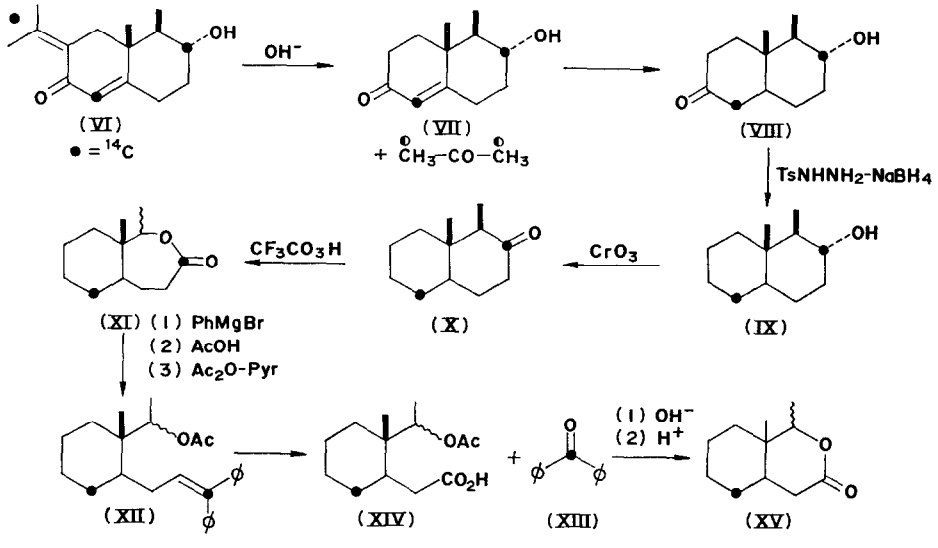
proton loss. The nature of the product would then be determined by the location of the proton that is abstracted, and this, in turn, may be controlled by the proximity and direction of a suitable proton acceptor in the enzyme. Loss of a proton from C-3 or C-14 leads to eudesmane type sesquiterpenoids, α- or β-selinene, while loss of the proton from C-1α



SCHEME 1. POSTULATED STEPS IN THE BIOGENESIS OF SESQUITERPENES OF THE COMPOSITAE.

or C-9α leads to the eremophilane class (Scheme 1). The latter process could give the eremophilane skeleton by a smooth sequence of steps from β-germacrene, without the postulated intervention of any uncharged, isolable eudesmanoid intermediates.

If this process were to occur with proton loss from C-1α, the product would have the structure of eremophilene. Eremophilene has been isolated, not only from *P. hybridus*, but also from a number of other species producing oxygenated eremophilane sesquiterpenoids, and it is an attractive hypothesis to view eremophilene as the common progenitor of such compounds.



In the experiments now described, the incorporation of [2-^{14}C]mevalonate into petasin (I, isolated as isopetasol) was initially studied. Two of the postulated hydrogen shifts in the scheme outlined above were then tested with the aid of [3*R*,4*R*-4-^{3}H]mevalonate. The label in this compound is retained in the biogenesis of *trans,trans*-farnesyl pyrophosphate.[10] This should give rise to β-germacrene labelled at 1α-H, 5α-H and 7α-H. Of these, 1α-H should be lost, and 5α-H should undergo a shift to 4α-H in the formation of eremophilene. This, in turn, should lead to petasin with tritium label at the 4α and 7α positions but without label at C-1.

RESULTS AND DISCUSSION

[2-^{14}C-3*R*,*S*]Mevalonolactone (MVAL) was fed to actively growing leaves of *P. hybridus*, and the plants were allowed 48 hr to metabolize the precursor. The leaves so treated were then extracted with isopropanol, and the petasin esters were isolated as a crude mixture by gel chromatography.[11,12] Petasin- and isopetasin-type esters yielded isopetasol (VI) as the common product after alkaline hydrolysis. This hydrolysis step was required to remove the acyl moieties, which had incorporated considerably more radioactivity than the sesquiterpenoid portion of the molecule. The isopetasol was purified to constant specific activity by repeated chromatography (Table 1). Incorporation into the sesquiterpenoid moiety of the esters was estimated as 0·005% of the radioactivity of the racemic precursor, after adjustment for losses in purification. These procedures were repeated in

[10] J. W. Cornforth, R. H. Cornforth, G. Popják and L. Yengoyan, *J. Biol. Chem.* **241**, 3970 (1966).
[11] J. Ellingboe, E. Nyström and J. Sjövall, *J. Lipid Res.* **11**, 266 (1970).
[12] C. J. W. Brooks and R. A. B. Keates, *J. Chromatog.* **44**, 509 (1969).

labelling experiments using the flowers of *P. hybridus*. After 24 hr metabolism of the precursor, 0·018% of the total radioactivity was recovered as isopetasol.

TABLE 1. PURIFICATION OF ISOPETASOL FROM 2-^{14}C MEVALONOLACTONE

Step	Recovery	(mg)	Specific activity (dpm/μmol)
From leaf feeding			
1 Hydrolysis of petasin esters	Isopetasol	55	—
2 N.1114-50%-LH20/benzene*, SEV 115–120	Isopetasol	44·9	35·2
3 Acetylation	Isopetasyl acetate	52	—
4 N.1114-50%-LH20/benzene, SEV 58–61	Isopetasyl acetate	49·2	31·1
5 N.1114-50%-LH20/methanol, SEV 68–70	Isopetasyl acetate	47·3	29·3
6 Hydrolysis	Isopetasol	42·5	—
7 N.1114-50%-LH20/methanol, SEV 64–66	Isopetasol	37·5	26·3
8 Silica gel/diethyl ether	Isopetasol	33·0	27·2
9 N.1114-50%-LH20/benzene, SEV 115–120	Isopetasol	30·0	27·8 ±3% S.E.M.
From flower feeding			
1 Hydrolysis of petasin esters	Isopetasol	36·6	—
2 N.1114-50%-LH20/benzene, SEV 115–120	Isopetasol	25·8	53·9
3 Acetylation	Isopetasyl acetate	30·3	—
4 N.1114-50%-LH20/methanol, SEV 68–70	Isopetasyl acetate	29·2	38·1
5 Hydrolysis	Isopetasol	23·2	—
6 N.1114-50%-LH20/methanol, SEV 64–66	Isopetasol	22·2	37·9
7 N.1114-50%-LH20/benzene, SEV 115–120	Isopetasol	21·1	37·4 ±3% S.E.M.

* N.1114-50%-LH20—modified Sephadex dextran gel.[11,12]

Isopetasol labelled by [2-^{14}C]MVAL was then degraded to determine the location of radioactivity in the eremophilane skeleton. Prolonged base treatment of isopetasol (VI) removed the isopropylidene side-chain as acetone by a retroaldol reaction:[2] conditions were devised affording desisopropylideneisopetasol (VII) in 75% yield and acetone in *ca.* 60% yield. The acetone was further degraded by the iodoform reaction. The radioactivity of the iodoform recovered indicated that one-sixth of the total radioactivity was located in each of the methyl groups at C-12 and C-13 of isopetasol, and the remaining two-thirds was found in desisopropylideneisopetasol (VII) (Table 2).

Desisopropylideneisopetasol (VII) was hydrogenated to give the ketoalcohol (VIII), which was then reduced to the saturated alcohol (IX) by treatment with *p*-toluenesulphonylhydrazine and sodium borohydride.[13] The ketone (X) was obtained by Jones oxidation, and converted to the ϵ-lactone (XI) (mixture of epimers at C-4) by Baeyer–Villiger oxidation. The ϵ-lactone was converted to the acetoxy diphenylethylene (XII).

[13] L. CAGLIOTI, *Tetrahedron* **22**, 487 (1966).

Oxidative cleavage of this product yielded benzophenone-(XIII) containing C-3 of the original isopetasol. The acetoxy carboxylic acid (XIV) was converted to the δ-lactone (XV), which contained the remaining 11 carbon atoms of desisopropylideneisopetasol. One-third of the radioactivity of isopetasol was located at C-3 (Table 3).

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN ISOPROPYLIDENE SIDE CHAIN OF ISOPETASOL

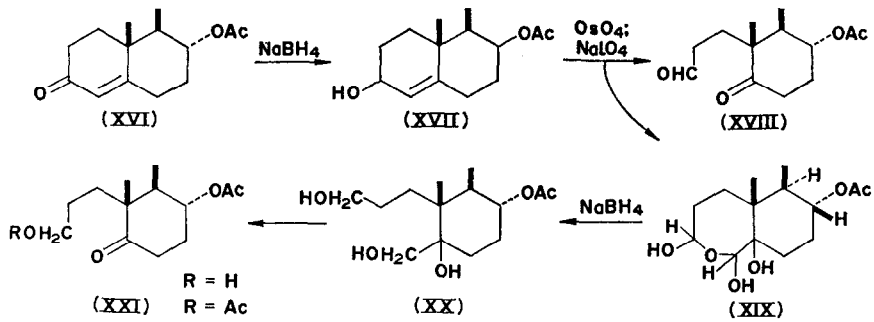
Radioactivity (dpm/μmol)	Isopetasol (VI)	Desisopropylideneisopetasol (VII)	Iodoform
Leaf feedings	27·8 ± 0·4	17·9 ± 0·3	5·1 ± 0·2
	100%	64·5%	18·5%
Flower feeding	37·2 ± 0·6	24·4 ± 0·4	6·4 ± 0·3
	100%	65·5%	17%
Theoretical	100%	67%	17%

Desisopropylideneisopetasyl acetate (XVI) was reduced to the allylic alcohol (XVII), which was treated with osmium tetroxide and sodium periodate. The acetoxy ketoaldehyde (XVIII), lacking only C-9 of desisopropylideneisopetasol, was isolated in poor yield (15%).

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN DESISOPROPYLIDENEISOPETASOL

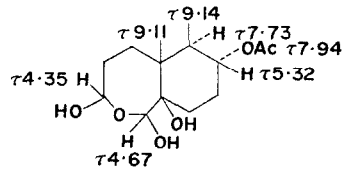
Compound	Radioactivity (dpm/μmol)	Actual (%)	Theory (%)
Desisopropylideneisopetasol (VII)	10·2 ± 0·2	65	67
Benzophenone (XIII)	5·0 ± 0·1	32	33
Acetoxy carboxylic acid (XIV)	5·2 ± 0·1	33	33
Acetoxy ketoaldehyde (XVIII)	5·25 ± 0·15	33·5	33
Acetoxy ketoalcohol (XXI)	5·3 ± 0·15	34	33

The major product (50%) was tentatively identified as the acetoxy-*bis*-hemiacetal (XIX), which resisted further oxidative cleavage by both periodic acid and sodium bismuthate.



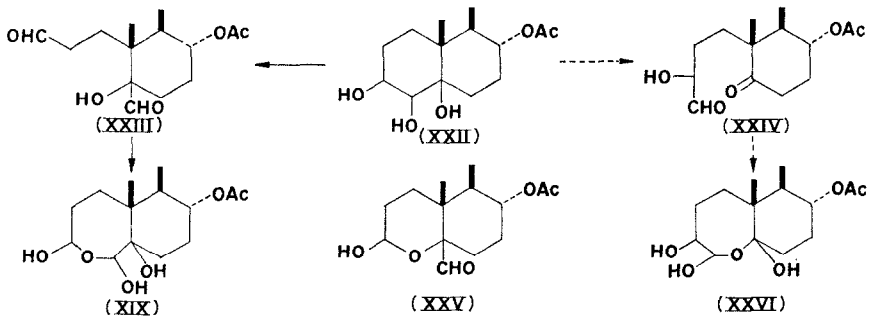
Reduction with sodium borohydride gave the acetoxy triol (XX), which was readily cleaved by periodic acid to give the acetoxy ketoalcohol (XXI). C_1-fragments (from C-9) were not recovered, but both the acetoxy ketoaldehyde (XVIII) and the acetoxy ketoalcohol (XXI) contained the remaining eleven carbon atoms of desisopropylideneisopetasol, with only one-half of its radioactivity (Table 3).

The structure of (XIX) followed from the NMR and MS data. 100 MHz NMR gave signals (Scheme 2) interpretable as indicated in the assignments to formula (XIX). The high field region of the spectrum suggested that the structure of ring *A* was intact. This was further confirmed by spin-decoupling experiments, and correlation of these results with data for isopetasyl acetate. Four additional protons in the complex region ascribed to ring methylene resonance (8·2–8·6 τ) suggested that a part of ring *B* was also unchanged. The Lemieux–Johnson reagent[14] would be expected to yield, via the osmate ester, an acetoxytriol (XXII). Periodate cleavage would then give a product such as XXIII or XXIV.



SCHEME 2. 100 MHz NMR DATA ($CDCl_3$) FOR THE MAJOR PRODUCT (XIX) OF OXIDATIVE CLEAVAGE OF ALLYLIC ALCOHOL (XVII).

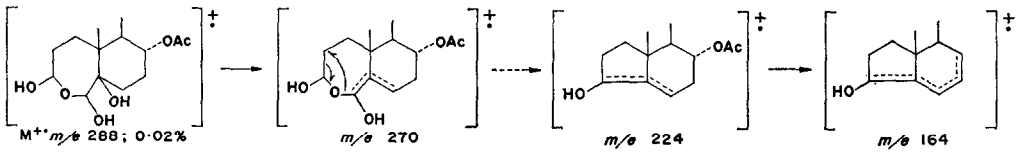
The relative intensity of IR absorption bands at ν_{max} 1750 and 1255 cm^{-1}, the latter being the more intense, suggested that these derived solely from the acetate group, and that carbonyl absorption from other sources was absent. This excluded the possibility that an aldol-type condensation had occurred. Mono hemiacetals (as XXV) were also excluded by



the IR absorption data, and by the lack of NMR signals due to allylic protons, hydrogen α- to carbonyl groups, or aldehyde protons (down to $\tau = -5{\cdot}0$). Only the *bis*-hemiacetals (XIX) and (XXVI) were in accord with spectroscopic evidence. Strong O–H stretching absorption at 3400 cm^{-1}, and a complex series of bands in the range 1140–960 cm^{-1} ascribed to C–O stretching modes, supported this interpretation.

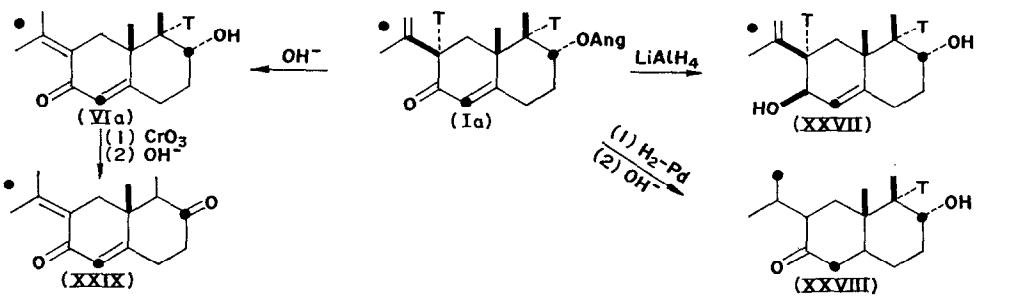
Exchange with D_2O gave further evidence from the NMR spectrum that prompted the assignment of structure (XIX). After exchange, the signal at $\tau = 7{\cdot}52$ disappeared, and that at $\tau = 4{\cdot}67$ collapsed to a sharp singlet, consistent with the isolated carbinol system as in (XIX). Irradiation at $\tau = 8{\cdot}5$ sharpened the signal at $\tau = 4{\cdot}35$, indicating the adjacency of this proton to ring methylene. The low field of this signal was inconsistent with a simple carbinol as in (XXV), but was compatible with the hemiacetal (XIX).

[14] R. PAPPO, D. S. ALLEN, JR., R. U. LEMIEUX and W. S. JOHNSON, *J. Org. Chem.* **21**, 478 (1956).



SCHEME 3. POSTULATED FRAGMENTATION MODE OF MAJOR PRODUCT (XIX) OF PERIODATE-OsO_4 CLEAVAGE OF COMPOUND (XVII).

The MS could be interpreted (Scheme 3) by postulating loss of water as a primary fragmentation process. The peak at *m/e* 224 could be formulated as $[M-18-46]^{+\cdot}$ ascribed to the loss of formic acid as a second fragmentation. Loss of the elements of acetic acid would then account for the intense peak at *m/e* 164. With foreknowledge of their expected location, the molecular ion, *m/e* 288, and $[M-18]^{+\cdot}$ were just discernible.



Attention was next given to the incorporation of tritium-labelled mevalonate. *P. hybridus* flowers, which had been shown to give better incorporation than leaves in ^{14}C experiments, were fed with [2-^{14}C-3*R*,4*R*-4-^{3}H] MVAL (together with the inactive enantiomer) and allowed to metabolise the precursor for 48 hr. The petasin esters were isolated as before, but were not hydrolysed to isopetasol, because of the susceptibility of petasin to exchange (by enolisation) of any tritium that might have been present at C-1 or C-7α.

TABLE 4. INCORPORATION OF [2-^{14}C-4-^{3}H] MEVALONOLACTONE INTO PETASIN DERIVATIVES

Compound	Tritium: ^{14}C ratios correlated with expected proton eliminations $^{3}H:^{14}C$ Measured	Normalized	Tritium labelled hydrogen lost by exchange or elimination
MVAL	5·9:1	3:3	—
Diol XXVII	3·8:1	1·9:3	1-H
Ketol XXVIII	2·6:1	1·3:3	1-H, 7α-H
Isopetasol VIa	2·3:1	1·2:3	1-H, 7α-H
Isopetasone XXIX	0·3:1	0·2:3	1-H, 7α-H, 4α-H

Petasin was separated from isopetasin and *S*-isopetasin by TLC, and was immediately treated with lithium aluminium hydride, to remove the highly radioactive angelic acid moiety, and to protect the molecule against further risk of hydrogen exchange. After purification of the diol (XXVII) by gel chromatography, the ratio of tritium to ^{14}C was measured and compared with that present in the mevalonolactone (Table 4). A further sample of petasin was hydrogenated, to avoid further exchange by preventing enolisation at C-1. After hydrolysis to remove angelic acid, the saturated ketol (XXVIII) was purified and the

ratio of tritium to ^{14}C was determined. A third sample of petasin was hydrolysed directly, and the isopetasol obtained was oxidised with Jones' reagent to give isopetasone (XXIX), which was then treated with base to allow exchange of any tritium located at C-4α. The tritium to ^{14}C ratios in isopetasol and isopetasone were then determined.

TABLE 5. SALIENT FEATURES OF MS AND IR SPECTRA, TOGETHER WITH RETENTION INDEX VALUES, FOR COMPOUNDS STUDIED

Compound	$M^{+\cdot}$		Base peak	ν_{max}* (cm^{-1})			Retention index	
	m/e	(%)	*m/e*	$\nu_{C=O}$		ν_{O-H}	Temp.	I(OV-I)
Isopetasol (VI)	234	(73)	161	1680s	1620w	—	162	1965
Isopetasyl acetate	276	(30)	161				180	2075
Desisopropylidene isopetasol (VII)	194	(37)	176	1680s	1620w	—	150	1770
Keto alcohol (VIII)	196	(0·8)	55	1710s		3500m	146	1690
Alcohol (IX)	182	(3)	96			3450m	119	1450
Ketone (X)	180	(16)	108	1710s			119	1430
ε-Lactone (XI)	196	(2)	81	1735s			140	1670 1675
Acetoxy diphenyl ethylene (XII)	376	(2)	115	1730s			226	2675 2685
Acetoxy carboxylic acid (XIV) (as methyl ester)	(196)†	(6)	95	1735s			140	1655 1675
δ-Lactone (XV)	182	(3)	96	1735s			140	1570 1580
Desisopropylidene isopetasyl acetate (XVI)	236	(0·5)	176	1735s 1625m	1675s		162	1850
Allylic alcohol (XVII)	238			1730s	1655w	3450m	162	1800
Acetoxy keto aldehyde‡ (XVIII)	240			1745sh 1710s	1735s		—	—
Acetoxy bis hemiacetal (XIX)	288	(0·02)	43	1730s		3400m	162	1915
Acetoxy ketol (XXI)	not recorded				not recorded		162	1760
(as diacetate)	284	(0·05)	124		not recorded		162	1900
Acetylenic ketone§ (XXX)	(absent)		124	1735s	1710s	3300m	140	1625
Diketone (XXXI)	254	(0·8)	124	1735s	1710s		150	1770

* Liquid film or Nujol mull.
† $[M-60]^{+\cdot}$.
‡ IR data recorded for CCl_4 solution: ν_{C-H} 2820, 2710 cm^{-1}.
§ $\nu_{C\equiv C}$ 2140 cm^{-1} (weak).

These results (Table 4) indicated clearly the location of one tritium at C-4α of petasin, confirming the participation of the 1,2-hydrogen shift in the migration of the angular methyl group. The results for labelling at C-1 and C-7α are less clear-cut. There is some risk of loss (by enolisation) of specifically labelled tritium at these positions: moreover, there is evidence of a degree of random labelling. The data are, at best, consistent with the absence of tritium from C-1 and with its presence at C-7α: it would be unwise to place too much reliance on the results for these positions. It would be preferable to confirm the presence of tritium at C-7α and its absence at C-1 through studies of a compound in which exchange is not possible, as in the case of eremophilene (Scheme 1), but this has not yet been attempted.

The interesting suggestion has recently been made[15] that the eremophilane skeleton—and

[15] D. J. DUNHAM and R. G. LAWTON, *J. Am. Chem. Soc.* **93**, 2075 (1971).

particularly that of nootkatene—may be formed in nature by a sequence of rearrangements via two *spiro*-intermediates, rather than by methyl group migration. Either process would give rise to the same distribution of ^{14}C from [2-^{14}C]MVAL. On the other hand, it is not clear whether the route involving *spiro*-intermediates would require the shift of a hydrogen atom from C-5α to C-4α, the occurrence of which was indicated by the tritium-labelling experiment mentioned above. A distinction between the alternative pathways should, of course, be possible through the incorporation of mevalonic acid suitably labelled with ^{14}C, e.g. at C-3.

EXPERIMENTAL

Chemicals. [2-^{14}C-3*RS*]Mevalonolactone (sp. act. 5·4 and 6·4 mCi/mmol) and [3*R*,4*R*-4-^{3}H + 3*S*,4*S*-4-^{3}H]-mevalonolactone (sp. act. 116 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. Doubly-labelled material was obtained by mixing samples.

Plant material. P. hybridus plants were taken from a site where they were growing wild, near Dumbarton, Dunbartonshire. Rhizomes were collected during the winter, and transplanted in greenhouses in the Botany Department facility at Garscube. Flowers were obtained by pre-selecting rhizomes with fully developed buds.

Feeding. Labelled mevalonolactone* was administered in aqueous solution (25 μCi/ml), using a cotton wick threaded through the petiole, dipping into a small vial (capacity 0·5 ml) attached to the petiole by self adhesive plastic tape. 90 μCi of [2-^{14}C]MVAL was fed to 14 leaves (180 g fr. wt); 15 μCi of [2-^{14}C]-MVAL was fed to 3 flowers (26·1 g fr. wt), and 25 μCi + 150 μCi of [2-^{14}C-4-^{3}H]MVAL was fed to 5 flowers (*ca.* 40 g fr. wt).

Extraction. The leaves (or flowers) were homogenized and extracted 3× with cold isoPrOH (400 ml). The combined extracts were evaporated to an oil.

Chromatography. Three columns were employed, using the modified dextran gel N.1114-50%-LH20.[12] Benzene, benzene–isoPrOH (3:1, v/v) and MeOH were used as liquid phases, for straight-phase, gel filtration and reversed-phase partition chromatography, respectively.

Methods of analysis and characterization. GLC was carried out with a Varian Aerograph Model 204 instrument fitted with 2 glass columns (3 mm i.d.): one (3 m) packed with 1% OV-1 on Gas Chrom P (acid-washed and silanized), 100–120 mesh, the other (2·1 m) with 0·5% XE-60 on the same support. GC–MS was carried out with 1·8 m or 3 m columns (1% OV-1) in the LKB 9000 instrument: the electron energy was normally 20 eV. IR data were recorded using the Perkin–Elmer 257 or Unicam SP 200 instruments. NMR spectra were recorded at 100 MHz with the Varian HA-100 instrument. Salient features of the spectrometric and gas chromatographic data are cited in Table 5. Radioactivity was measured by liquid scintillation counting, using the Philips Model PW-4530 liquid scintillation analyser.

Hydrolysis of petasin esters and acetylation of isopetasol. The petasin esters (120 mg) in EtOH (4 ml) and 3·5 M KOH (0·5 ml) were refluxed for 30 min, and isopetasol was recovered by extraction with benzene. After purification by gel chromatography, isopetasol (45 mg, m.p. 127°) was treated overnight with pyridine (0·25 ml) and Ac_2O (0·5 ml). Isopetasyl acetate (47 mg, m.p. 85°) was purified by gel chromatography.

Retroaldol reaction and iodoform reaction. Isopetasol (29 mg) in MeOH (3 ml) and 3·5 M KOH (2·8 ml) was degassed under vacuum, and the mixture refluxed for 18 hr under N_2. MeOH and the acetone produced were then distilled into a chilled tube. 1 M KOH (4 ml) was added to the distillate, followed by I_2 (50 mg). Iodoform (*ca.* 25 mg) precipitated immediately, and was recrystallized from 80% EtOH. Desisopropylideneisopetasol was extracted with benzene—EtOAc (1:1, v/v). After gel chromatography, pure desisopropylideneisopetasol (18·2 mg, m.p. 103°, λ_{max} (EtOH), 238 nm, ϵ 11 000) was obtained (Found: C, 74·03; H, 9·58. $C_{12}H_{18}O_2$ requires: C, 74·19; H, 9·34%). NMR data (τ values) ($CDCl_3$): 4·29 *br* (9-H), 6·46 *m* (3-H), 7·6 *m* (7-H_2), 8·3 *m* (6-H_2), 8·92 *s*+*d* (15-H_3, 14-H_3). The radioactive sample was diluted with unlabelled carrier at this stage to facilitate further degradation.

Hydrogenation of desisopropylideneisopetasol. Desisopropylideneisopetasol (31·8 mg); was hydrogenated for 30 min in EtOAc (2·5 ml) with 10% Pd–C (7 mg) as catalyst. The ketoalcohol (VIII; 32·0 mg) was recovered by evaporation of the solvent.

Reduction of ketoalcohol (VIII) *to alcohol* (IX).[13] The saturated ketoalcohol (VIII; 32 mg) was treated with *p*-tosylhydrazine (32 mg; 1·05 equivalents) and refluxed in MeOH for 2 hr. After cooling, $NaBH_4$ (40 mg) was added over 90 min and the mixture refluxed for 5 hr to decompose the tosylhydrazide. H_2O was added, and the saturated alcohol (IX) extracted using benzene–EtOAc (1:4, v/v).

Jones' oxidation of alcohol (IX). The product from the previous step was taken without purification, and

* The lactone and free acid were equally effective as precursors, but uptake of labelled sample was more rapid with the lactone.

was oxidized in acetone (1 ml) with Jones' reagent[16] (75 μl; 250 μmol). After 2–3 min, H_2O was added, and ketone (X) extracted with benzene. The saturated ketone (X; 9 mg), ν_{max} 1710 cm^{-1}, with a pronounced odour of mint, was purified by reversed-phase column chromatography.

Baeyer–Villiger oxidation of saturated ketone (X). Peroxytrifluoroacetic acid was prepared by adding trifluoroacetic anhydride (250 μl) to a suspension of 90% H_2O_2 (40 μl) in CH_2Cl_2 (250 μl) at 0°.[17] A solution of the ketone (X; 9·0 mg) in CH_2Cl_2 (0·25 ml) was stirred with freshly dried Na_2HPO_4 (35 mg).[18] CF_3CO_3H (30 μl) was added over 20 min, with vigorous stirring. Stirring was continued for 1 hr, the mixture diluted with CH_2Cl_2 (2 ml) and the solid removed by centrifugation. The organic extract was evaporated to yield ϵ-lactone (XI; 9·6 mg; ν_{max} 1735 cm^{-1}).

Barbier–Wieland degradation[19–21] *of ϵ-lactone* (XI). The ϵ-lactone (XI; 9·6 mg; 49 μmol) was treated with an Et_2O solution of phenylmagnesium bromide (0·6 mmol) and stirred under reflux overnight. The adduct was treated with ice H_2O containing H_2SO_4, and the organic product was extracted with Et_2O. The aromatic oil obtained after evaporation of the Et_2O was warmed *in vacuo* to remove biphenyl. The product was refluxed in 80% HOAc (1 ml) for 8 hr, the acid neutralized, and the organic product was extracted in benzene. Evaporation of the benzene gave an oil, which was treated with dry pyridine (0·1 ml) and Ac_2O (0·3 ml). After 12 hr, EtOH was added, and the reagents removed by evaporation. The product was purified by straight-phase gel chromatography (N.1114-50%-LH20/benzene) and the acetoxydiphenylethylene (XII; 10·2 mg) was obtained as a mixture of epimers [ν_{max} (cm^{-1}) 3040 (w) 1730 (s) 1600 (m) 1500 (m) 815 (s), λ_{max} (EtOH) 251 nm (ϵ_{max} 11 500); NMR data (τ values): ($CDCl_3$): 2·7–3·0 (phenyl protons), 4·1 br (2-H_1), 5·1 m (4-H_1), 8·10 (4-acetate, $H_{1.5}$), 8·40 (4-acetate, $H_{1.5}$), 9·03 d (14-H_3) (J = 6) 9·08 s (15-$H_{1.5}$), 9·29 s (15-$H_{1.5}$)].

Ruthenium tetroxide cleavage of acetoxy diphenylethylene (XII).[20,22] Ruthenium tetroxide was prepared by stirring ruthenium dioxide (9·6 mg) in H_2O (1 ml) containing $NaIO_4$ (50 mg) for 30 min. 50 μl of this solution (*ca.* 600 μg RuO_4) was added to a solution of the acetoxydiphenylethylene (XII; 10·2 mg) in acetone (1 ml), and finely-powdered $NaIO_4$ was added in portions during 4 hr with continuous stirring. H_2O (0·2 ml) was added over the same period. After destruction of excess reagent with isoPrOH, acetone was added, and $NaIO_3$ and RuO_2 were removed by centrifugation. After evaporation, the organic products were partitioned between benzene and 0·5 M Na_2CO_3. Benzophenone (XIII) was recovered from the benzene extract. After acidification of the Na_2CO_3 layer, the acid products of the cleavage reaction were recovered by extraction with EtOAc. The acetoxy carboxylic acid (XIV) was methylated with CH_2N_2 for purification by straight-phase chromatography. The product gave the following NMR data (τ values) ($CDCl_3$): 5·06 q (4-H_1) (J = 6·5 Hz), 6·39 s (methyl ester), 7·62 m (10-H_1), 8·01 s (4-acetate), 8·86 d (14-$H_{1.5}$) (J = 6·5 Hz), 8·92 d (14-$H_{1.5}$), 9·00 s (15-$H_{1.5}$) 9·20 s (15-$H_{1.5}$).

Formation of δ-lactone (XV). The methyl ester of acetoxy-acid (XIV) was hydrolysed in ethanolic KOH for 40 min. The mixture was then acidified to pH 3, and extracted with EtOAc to give a colourless oil (3·5 mg), identified as the δ-lactone (XV).

Borohydride reduction of desisopropylideneisopetasyl acetate (XVI). A solution of desisopropylideneisopetasyl acetate (XVI; 10·4 mg) in EtOH (1 ml) was treated with $NaBH_4$ (3 mg). After stirring for 1 hr. H_2O was added and the product was extracted with EtOAc–benzene (3:1, v/v). The allylic alcohol (XVII) was recovered by evaporation of the solvent.

Cleavage of (XVII) *with Lemieux–Johnson reagent.*[14,23] The allylic alcohol (XVII) was dissolved in a mixture of dioxan (0·3 ml) and H_2O (0·1 ml). OsO_4 (2·2 mg) was added and the mixture was stirred until the crystals had dissolved with the formation of a brown suspension. $NaIO_4$ (4 mg) was added and the mixture became decolorized. $NaIO_4$ was added at intervals (to a total of 32 mg) whenever the brown colour returned. After 30 hr, H_2O (0·1 ml) was added and after 48 hr, the brown colour ceased to form. The organic products were extracted in EtOAc–benzene (3:1, v/v). Straight-phase gel chromatography gave the acetoxy ketoaldehyde (XVIII) in low yield (1·2 mg). The major product of the reaction was also recovered, and identified as acetoxy *bis*-hemiacetal (XIX) mainly on the evidence given (Schemes 2 and 3). Further chemical evidence for the structure (XIX) was provided by the succeeding reactions.

Borohydride reduction of acetoxy-bis-hemiacetal (XIX). Acetoxy-*bis*-hemiacetal (XIX) was treated with $NaBH_4$ (2 mg) in ethanol (1 ml). After 12 hr, 2 mg more of $NaBH_4$ was added; after 24 hr, ethylene glycol (10 μl) was added to destroy the borate complex formed by acetoxy triol (XX). After extraction, acetoxy triol (3·0 mg) was recovered.

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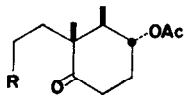
Periodate cleavage of acetoxy triol (XX).[24] Acetoxy triol (XX) in Et_2O was treated with a saturated solution of HIO_4 in Et_2O until iodic acid ceased to precipitate. Excess reagent was destroyed with ethylene glycol, as $Na_2S_2O_5$ caused loss of sample by a side reaction. After centrifugation to remove HIO_3, the organic products were purified by straight-phase gel chromatography. The acetoxy ketol (XXI) was acetylated and identified by MS correlation with analogous compounds.



(XXX) R = C≡CH

(XXXI) R = CO≡CH_3

Preparation of acetylenic ketone (XXX) *and diketone* (XXXI). Structural analogues of acetoxy ketol (XXI) were prepared in the course of an attempt to isolate C-9 of isopetasol by the Eschenmoser fragmentation reaction.[25,26] Desisopropylidene-isopetasyl acetate (19 mg) was epoxidized with H_2O_2 (75 μl) in a solution of Na_2CO_3 (17 mg) in 70% EtOH (2 ml). After continuous stirring for 5 hr, 9,10-epoxydesisopropylideneisopetasyl acetate (XXXII; 15·8 mg) was recovered. This was treated with tosylhydrazine (16 mg) in EtOH (1 ml) and heated to 50° for 30 min. The acetylenic ketone (XXX; 13·2 mg) was recovered and characterized spectroscopically. High yield in this reaction was dependent on the use of exactly equal molar proportions of substrate and tosylhydrazine. The acetylenic ketone (13·2 mg) was dissolved in trifluoroacetic acid (0·2 ml) in the presence of H_2O (10 μl) and red HgO (0·5 mg). After shaking the suspension at room temperature for 1 hr, diketone (XXXI; 12·4 mg) was recovered, and characterized spectroscopically. The yield of (XXXI) from desisopropylideneisopetasyl acetate was about 60%. This reaction sequence failed when no product could be characterized after iodoform or bromoform degradation of the diketone (XXXI).

The three products of degradation of ring B (XXI, XXX, XXXI) underwent a facile McLafferty rearrangement during MS: this, coupled with the elimination of acetic acid, gave rise to prominent base peaks at *m/e* 124 for each of these compounds.

Acknowledgements—We thank Dr. A. M. M. Berrie (Botany Department) for his advice and assistance in the cultivation of *P. hybridus*, Dr. J. A. Zabkiewicz for his helpful interest in the early stages of this work, Miss Juliet Johnson for GC–MS runs, and Mr. J. Gall for small-scale NMR measurements. This work was carried out during the tenure of a Demonstratorship (R.A.B.K.). The LKB 9000 gas chromatograph–mass spectrometer was provided by S.R.C. grant No. B/SR/2398.

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