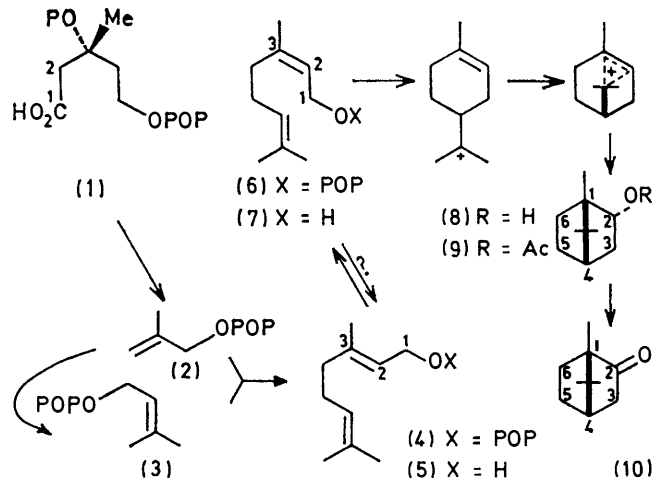
# Biosynthesis. Part XIX.† [1] Concerning the Biosynthesis of (−)-Camphor and (−)-Borneol in *Salvia officinalis*

By **A. R. Battersby,* D. G. Laing,** and **R. Ramage,** The Robert Robinson Laboratories, University of Liverpool and the University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW

*Salvia officinalis* is selected after extractions and tracer experiments as a suitable species for biosynthetic work on bicyclic monoterpenes. [2-$^{14}$C]Geraniol is synthesised and is shown to be incorporated specifically by *S. officinalis* plants into camphor and borneol by unambiguous degradation of the isolated terpenes. Comment is made on results which emphasise the dangers of reliance on g.l.c. purification of terpenes for radio-tracer work.

THE present studies are concerned with the classical monoterpenes camphor (10) and borneol (8). Our present chemical understanding owes much to the extensive research on these bicyclic systems carried out from the mid-nineteenth century to the present day.[2] Yet despite their great chemical interest, virtually nothing was known at the outset of our work (1967) about the biosynthesis of camphor and borneol.[3] Attractive biosynthetic schemes for monoterpenes had been suggested,[4] however, in 1953. Scheme 1 brings these speculations up to date by incorporating firm knowledge of the early stages leading from structure (1) *via* (2) and (3) to geranyl (4) and neryl (6) pyrophosphates.[5] The proven formation [6] of the neryl from the geranyl system ‡ is also included.

The planning of the current work took into account our wish to determine at a later stage the stereochemical changes which occur as the cage structures (8) and (10) are formed. This will involve regiospecific and stereospecific labelling of suitable precursor(s) with tritium; [7] geraniol (5) and nerol (7) were selected for this purpose. The first step was thus to establish the incorporation of $^{14}$C-labelled geraniol or nerol into the monoterpenes (8) and (10) without randomisation of the label.




P = phosphate, POP = pyrophosphate

SCHEME 1 Hypothetical pathway to camphor and borneol

† Earlier parts of this series have appeared under the general title ' Alkaloid Biosynthesis.'

‡ Scheme 1 is not intended to imply a *direct* conversion (4) ⟶ (6).

[1] Part XVIII, A. R. Battersby, R. B. Herbert, E. McDonald, and R. Ramage, *J.C.S. Perkin I*, 1972, 1741.

[2] J. L. Simonsen and L. N. Owen, ' The Terpenes,' 2nd edn., Cambridge University Press, 1949, vol. II; A. Pelter and S. H. Harper in ' Rodd's Chemistry of Carbon Compounds,' 2nd edn., ed. S. Coffey, Elsevier, Amsterdam 1969, vol. IIC., p. 136; J. A. Berson in ' Molecular Rearrangements,' ed. P. de Mayo, Interscience, New York, 1963, vol. I, p. 111.

[3] W. D. Loomis, in ' Terpenoids in Plants,' ed. J. B. Pridham, Academic Press, New York, 1967, p. 59.

[4] L. Ruzicka, *Experientia*, 1953, **9**, 357.

[5] J. H. Richards and J. B. Hendrickson, ' Biosynthesis of Steroids, Terpenes and Acetogenins,' Benjamin, New York, 1964, p. 173; G. Popják and J. W. Cornforth, *Biochem. J.*, 1966, **101**, 553, and references therein; G. P. Moss, *Chem. Soc. Specialist Periodical Reports, Terpenoids and Steroids*, 1971, **1**, 221, and references therein.

[6] M. J. O. Francis, D. V. Banthorpe, and G. N. J. Le Patourel, *Nature*, 1970, **228**, 1005; *cf.* A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni, and P. Loew, *Chem. Comm.*, 1968, 951.

[7] A. R. Battersby, *Accounts Chem. Res.*, 1972, **5**, 148.

A major problem [3] in studying monoterpene biosynthesis in higher plants is the difficulty of achieving incorporations of precursor into product which are high enough to allow proper degradative work. This problem probably arises because of barriers in the living organism which largely prevent exogenous labelled materials reaching the site where biosynthesis occurs (compartmentalisation). The selection of a co-operative plant is thus of decisive importance. Accordingly, the steam-volatile oils from a set of thirteen plants were screened by g.l.c. for their content of camphor, borneol, and bornyl acetate. On the basis of the results (Table 1), the following four plants were chosen for preparative extraction and for the preliminary tracer studies: *Artemesia californica*, *Picea albertiana conica*, *Picea pungence glauca*, and *Salvia officinalis*. Table 2 shows that workable quantities of camphor and borneol (or bornyl acetate) are present in these species. It is noteworthy that between the harvesting dates of June 3rd and 20th (Tables 1 and 2, respectively) the proportion of borneol in the oil from *Salvia officinalis* increased considerably relative to that of camphor, and this was particularly evident on the analytical g.l.c. trace. An even more dramatic change occurred in the composition of oil from *Rosmarinus officinalis* when extracted from plants at different times in the growing season (*cf.* refs. 3 and 20). The oil (June harvest) did not hold promise for the current work nor did it contain even traces of the substance which appeared as the major component in oil extracted from plants harvested in August. This material was isolated, was found by analysis and mass spectrometry to be of composition $C_{10}H_{14}O$, and was identified as verbenone (pin-2-en-4-one) (11) by direct comparison with authentic material. This ketone occurs in frankincense from *Boswellia carterii* [21] and in Spanish verbena oil,[22] but it had not previously been isolated from *Rosmarinus officinalis*. Other workers have recently reported its presence in this plant.[23]

TABLE 1

Examination of plants for monoterpenes by g.l.c.

| Plant | Reported content and sign of rotation [a,b] Camphor | Borneol | Bornyl acetate | Found in our specimens [c] Camphor | Borneol | Bornyl acetate |
|---|---|---|---|---|---|---|
| *Picea mariana nana* [8] | (±) 1% | | 37% | *d* | *d* | Present? |
| *Picea pungence glauca* [9] | Major | Minor | Minor | Large | Minor | Large |
| *Picea albertiana nana* [9] | Major | Minor | Minor | Large [e] | Minor [e] | Large [e] |
| *Picea sitchensis* [10] | (+) 17% | (+) Minor | (+) Minor | *d* | Minor | *d* |
| *Lavandula spica* [11] | 0—4% | 2·3—3·8% | | Trace | Minor | *d* |
| *Artemesia californica* [12] | (+) | | | Major | Minor | Minor |
| *Thuja occidentalis* [13] | (−) 2·5% | | (−) 5·9% | Minor | Trace | Minor |
| *Thuja orientalis* [14] | 5·6% | (−) 17·1% | | Trace | Major | *d* |
| *Thuja standishii* [15] | | (−) | Present | Trace | Minor | *d* |
| *Salvia officinalis* [16] (variegated) | 8·2% | 6·6% | 1·7% | Large | Large | Trace |
| *Chamaecyparus lawsonia* [17] | (+) 0·5% | (+) 11% | (+) 2—3% | Trace | Major | *d* |
| *Pinus sylvestris* [18] | (±) | (±) and (−) | Present | Trace | Minor | *d* |
| *Rosmarinus officinalis* [19] | Present | Present | | Minor | Absent | *d* |

[a] Percentages refer to total oil obtained from the plant. [b] Many other terpenoids usually present in addition to the three below. [c] Harvested 3rd June. [d] Not detected. [e] Our specimen was *Picea albertiana conica*.

TABLE 2

Preparative separation of some monoterpenes

| Plant [a] | Fresh wt. (g) | Camphor Rotn. and wt. (mg) [b] | Borneol Rotn. and wt. (mg) [b] | Bornyl acetate Rotn. and wt. (mg) [b] |
|---|---|---|---|---|
| *Artemesia californica* | 88 | Trace | Trace | (+) 13 |
| *Picea albertiana conica* | 120 | (+) 29 | Trace | (+) 36 |
| *Picea pungence glauca* | 300 | (−) 15 | Trace | (−) 30 |
| *Salvia officinalis* | 200 | (−) 3 | (−) 8 | Trace |

[a] Harvested 20th June. [b] Minimum values; trial runs showed some loss due to incomplete trapping from the g.l.c. gas stream.

[1-$^{14}$C]Geraniol was prepared by the Wittig method [24] from [1-$^{14}$C]bromoacetic acid and it was administered as an aqueous emulsion to shoots of the four plants listed in Table 2. Batches of shoots were harvested after 6, 11, 24, and 48 h and were worked up separately for camphor, borneol, and bornyl acetate with addition of the appropriate radio-inactive enantiomers of each as carrier materials. The terpenoid oils were then fractionated by preparative g.l.c. and strict precautions were

[8] A. C. Shaw, *Canad. J. Res.*, 1950, **28***B*, 268.
[9] M. V. Schauntz, *Planta Med.*, 1965, **13**, 369.
[10] E. von Rudloff, *Canad. J. Chem.*, 1964, **42**, 1057.
[11] P. Rovesti, *Riv. ital. essenzel profumi piante offic. olii vegetali saponi*, 1956, **38**, 341.
[12] D. V. Banthorpe and D. Baxendale, *Chem. Comm.*, 1968, 1553; *J. Chem. Soc.* (*C*), 1970, 2694.
[13] A. C. Shaw, *Canad. J. Chem.*, 1953, **31**, 277.
[14] V. N. Vashist, M. C. Nigam, K. L. Handa, and G. N. Gupta, *Indian Oil Soap J.*, 1963, **29**, 45.
[15] T. Nakatsuka and Y. Hirose, *J. Japan Forestry Soc.*, 1955, **37**, 496.
[16] C. H. Brieskorn and E. Wenger, *Arch. Pharm.*, 1960, **293**, 21.
[17] G. Kritchevsky and A. B. Anderson, *J. Amer. Pharm. Assoc.*, 1955, **44**, 535.
[18] I. I. Bardyshev and R. I. Livshitz, *Zhur. priklad. Khim.*, 1952, **25**, 1289.
[19] G. Pellini, *Ann. Chim. Applicata*, 1923, **13**, 97.
[20] D. V. Banthorpe and A. Wirz-Justice, *J. Chem. Soc.* (*C*), 1969, 541.
[21] E. Guenther, 'The Essential Oils,' Van Nostrand, New York, 1948.
[22] M. Kerschbaum, *Ber.*, 1900, **33**, 885.
[23] R. Granger, J. Passett, G. Arbousset, and J. P. Girard, *Compt. rend.*, 1970, **270***D*, 209.
[24] *Cf.*, A. R. Battersby, R. T. Brown, J. A. Knight, J. A. Martin, and A. O. Plunkett, *Chem. Comm.*, 1966, 346.

taken to avoid radioactive cross contamination of one fraction by another. At this stage, the monoterpenes (8)—(10) were chemically pure as judged by analytical g.l.c. and they were all encouragingly radioactive. They were, however, far from radiochemically pure; comment will be made later on this important aspect. Each monoterpene was converted into a crystalline derivative, the oxime or semicarbazone for camphor and the phenylurethane for borneol which was isolated directly or had been obtained by hydrolysis of the acetate. Purification of these products caused a sharp fall in radioactivity in all cases. Thus, the specific activity of bornyl phenylurethane as first prepared from bornyl acetate isolated from *Picea albertiana conica* was 6460 counts per 100 s mmol$^{-1}$, which had fallen to zero after three recrystallisations. Similarly, camphor from *Picea pungence glauca* initially gave a semicarbazone of activity 5760 counts per 100 s mmol$^{-1}$, which figure was reduced to 455 by recrystallisation. Only camphor and borneol from *Salvia officinalis* retained a satisfactory activity after rigorous purification of the crystalline derivatives (see Table 3), though the results for *Artemesia californica* indicated that further work with this plant was justified. No major differences were observed in the incorporations of activity into the purified derivatives obtained from 6 or 48 h feeding periods with *S. officinalis*, so harvesting after 29 h was adopted for the large-scale work.

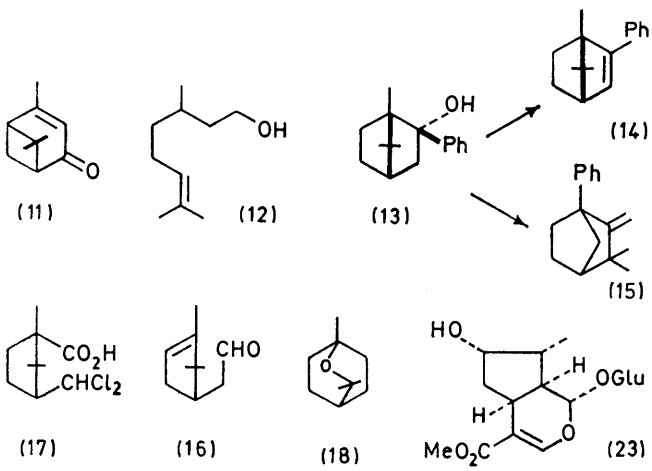



Table 3 shows the results obtained by feeding 0·39 mCi of [2-$^{14}$C]geraniol to *S. officinalis* plants followed by isolation of the monoterpenes (g.l.c.) and conversion of these materials into crystalline derivatives. Comparison of the apparent and true incorporation values again shows the dangers of reliance on g.l.c. purification for radio-tracer work in this field. Results similar to those in Table 3 have been obtained [25] with other monoterpenes extensively purified by adsorption chromatography and preparative t.l.c.; clearly, reliable incorporation values and degradative work can only be based on crystalline derivatives purified to constant activity.

TABLE 3

Tracer experiments on *S. officinalis* and *A. californica*

| | Apparent incorporation [a] (%) | | True incorporation [b] (%) | |
|---|---|---|---|---|
| Precursor | Camphor | Borneol | Camphor | Borneol |
| [1-$^{14}$C]-Geraniol [c] | | | $1 \times 10^{-3}$ | $3 \times 10^{-3}$ |
| [2-$^{14}$C]-Geraniol [c] | $2{\cdot}5 \times 10^{-3}$ | $120 \times 10^{-3}$ | $0{\cdot}5 \times 10^{-3}$ | $3{\cdot}3 \times 10^{-3}$ |
| [2-$^{14}$C]-Geraniol [d] | $4{\cdot}7 \times 10^{-2}$ | $3{\cdot}6 \times 10^{-2}$ [e] | Virtually radio-inactive | Radio-inactive |

[a] Determined on preparative g.l.c. fractions. [b] Determined on crystalline derivatives at constant specific activity. [c] Fed to *S. officinalis*. [d] Fed to *A. californica*. [e] From hydrolysis of bornyl acetate.

A similar experiment in which 0·39 mCi of [2-$^{14}$C]-geraniol was fed to *A. californica* plants showed this species to be valueless for the current project (Table 3). Banthorpe and Baxendale [12] have found, however, that plants of this species will incorporate [2-$^{14}$C]mevalonic acid [*cf.* (1)] to form radioactive (+)-camphor [0·002—0·032% incorp.; enantiomer of (10)] which was largely labelled at C-6 (73—83% of total).

The [2-$^{14}$C]geraniol used was prepared as earlier [24] from [2-$^{14}$C]bromoacetic acid with the modification that the intermediate methyl geranate was reduced by lithium aluminium monoethoxyhydride.[26] This yielded a purer product by appreciably lowering the amount of citronellol (3,7-dimethyloct-6-en-1-ol) (12) formed [27] when the reduction was carried out with lithium aluminium hydride.

With radiochemically pure (—)-[$^{14}$C]camphor (10) and (—)-[$^{14}$C]borneol (8) available, it was necessary to develop an unambiguous method for the isolation from these terpenes of the carbon atom at position 2, the expected site of labelling. The following routes were studied.

(a) via 2-*Phenylborneol* (13).—This alcohol is obtained [28] possibly together with some of its C-2 epimer [29] when camphor reacts with phenylmagnesium bromide, and it was hoped to obtain benzoic acid from it by oxidation.[30] Many experiments with permanganate under a wide range of alkaline conditions left compound (13) unchanged, and it could be recovered as such or largely as 2-phenylcamphene [28] (15) if the reaction mixture was acidified during work-up.

Since several ways for degradation of 2-phenylbornene (14) can be envisaged, dehydration of (13) to (14) over alumina and thermally in dimethyl sulphoxide [31] was studied. Both methods under the best conditions

[25] A. R. Battersby, R. Ramage, and D. A. Rowlands, in preparation.

[26] *Cf.*, R. S. Davidson, W. H. Günther, B. Lythgoe, and M. Waddington-Feather, *J. Chem. Soc.*, 1964, 4907.

[27] A. R. Battersby and R. J. Parry, unpublished work.

[28] N. C. Deno, J. J. Jaruzelzki, and A. Schriescheim, *J. Amer. Chem. Soc.*, 1955, **77**, 3044; D. Bernstein, *Annalen*, 1967, **710**, 98.

[29] *Cf.* D. F. MacSweeney and R. Ramage, *Tetrahedron*, 1971, **27**, 1481.

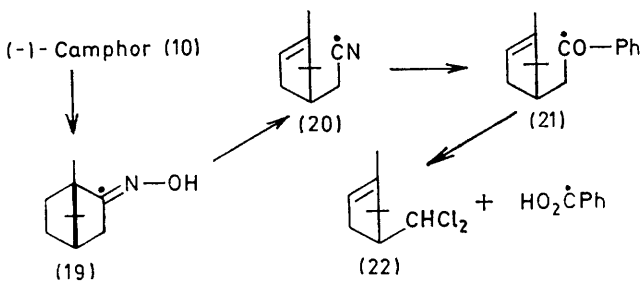
[30] A. R. Battersby, M. Hirst, D. J. McCaldin, R. Southgate, and J. Staunton, *J. Chem. Soc.* (*C*), 1968, 2163.

[31] V. J. Traynelis, W. L. Hergenrother, H. T. Hanson, and J. A. Valicenti, *J. Org. Chem.*, 1964, **29**, 123.

afforded a mixture of compounds (15) and (14) in proportions *ca.* 60 : 40, respectively (by n.m.r.). Heating compound (13) in dimethylformamide gave mainly (14) but in low yield.

(b) via α-*Campholenic Aldehyde* (2,2,3-*Trimethylcyclopent*-3-*enylacetaldehyde*) (16).—Photolysis of camphor is known [32] to yield the aldehyde (16) in yields >70%. However, treatment of the product (16) with phenylmagnesium bromide did not yield the desired material.

(c) via α-*Campholenic Nitrile* (2,2,3-*Trimethylcyclopent*-3-*enylacetonitrile*) (20).—A satisfactory degradative route (Scheme 2) was developed from this nitrile,[33]




SCHEME 2 Degradation of camphor

which is available by abnormal Beckmann rearrangement of camphor oxime (19) with hot phosphoric oxide [34] or, conveniently for small-scale work as here, with acetyl chloride. The nitrile was converted by phenylmagnesium bromide into the ketone (21), which is fully substituted α to the carbonyl group and carries two α'-hydrogen atoms. It was thus expected to be cleaved by treatment with base and carbon tetrachloride just as camphor is converted [35] under these conditions into the acid (17). This expectation was realised and benzoic acid was isolated; we have not attempted to characterise the other fragment, presumably (22).

The radioactive (−)-camphor obtained by feeding [2-$^{14}$C]geraniol to *S. officinalis* was taken through the sequence in Scheme 2; the benzoic acid so obtained carried (on a molar basis) 95% of the original activity. Jones oxidation of the (−)-borneol from the same feeding experiment afforded (−)-camphor, which was degraded in the same way. The activity of the final benzoic acid corresponded in this case to 82% of that in the camphor oxime which had been purified to constant specific activity. The amounts of camphor and borneol available for degradation did not allow the counting of many replicate samples and so a ' mean value ' and a ' standard deviation from the mean ' cannot be quoted. However, experience of the errors involved in counting other ' low-level ' samples indicate that the foregoing values will probably hold within ±10%.

These experiments demonstrate that *S. officinalis* plants specifically incorporate [2-$^{14}$C]geraniol into (−)-camphor (10) and (−)-borneol (8) such that the tracer is largely located at C-2 of both monoterpenes. This is in accord with Scheme 1. The way is now open for stereochemical studies of the biochemical ring-closure reactions leading to (10) and (8).

The incorporation has also been reported of labelled geranyl pyrophosphate (4) into cineole (18) without randomisation of the label in *Rosmarinus officinalis* plants [36] and into the monoterpenes of *Tanacetum*.[20] Degradations to determine the site(s) of labelling in the latter cases should afford results of considerable interest. In contrast to the sparse information available so far about the biosynthesis of the monoterpenes considered here, there is extensive knowledge of the biochemical pathway leading to the cyclopentane monoterpenes [37] and especially to loganin (23).

## EXPERIMENTAL

Most of the general chemical and radiochemical directions are given in ref. 38. In addition, i.r. spectra were recorded for liquid films unless otherwise stated; material on t.l.c. plates was detected by exposure to iodine vapour or by spraying with ethanolic phosphomolybdic acid followed by heating at 100°.

Analytical g.l.c. was carried out on a Perkin-Elmer F11 instrument with nitrogen as carrier gas and glass columns containing Carbowax 20M (5—10% w/w) on Chromosorb W (70—80 mesh). Perkin-Elmer F21 and Pye 105 instruments were used for preparative work, the Carbowax 20M being increased to 20—30% w/w.

*Screening of Plants for Terpenes.*—The plant material (30—80 g fresh wt.) was macerated for 2—3 min with methanol in a Waring blender; the suspension was filtered (Celite) and the filtrate was concentrated. Steam distillation of the residual solution, extraction of the distillate with methylene chloride, and evaporation of the extract gave an oil which was analysed by g.l.c. (Table 1).

*Preparative Separation of Terpenes.*—Plant material (100—300 g fresh wt.) was finely ground with liquid nitrogen and was then transferred frozen into a Soxhlet thimble and extracted with ether for 15 h. The extract, without concentration, was steam distilled for 2·5 h and the collected ether was used to extract the aqueous phase. The latter was then extracted thrice with fresh ether. Evaporation of the combined, dried ethereal solution through a column (6 × $\frac{3}{4}$ in, beads or helices) left an oil which was fractionated by preparative g.l.c. (Table 2).

The oil thus obtained from *Rosmarinus officinalis* harvested in August gave as the main component, verbenone (pin-2-en-4-one) (11), $\nu_{max.}$ 1680 and 1610 cm$^{-1}$; *m/e* 150 ($M^+$); τ 9·0 (3H, s), 8·5 (3H, s), 8·0 (3H, d), and 4·3 (1H, m), identical (g.l.c., i.r. and n.m.r. spectra) with authentic material.

*Reduction of Methyl* [2-$^{14}$C]*Geranate.*—A solution of anhydrous ethanol (0·045 ml) in ether (5 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (0·5 g) in ether (25 ml). After effervescence ceased, part of this solution (0·5 ml) at 5° was added to a stirred solution of methyl [2-$^{14}$C]geranate [24] (1·95 mCi; 109 mg) in ether

[32] R. Srinivasan, *J. Amer. Chem. Soc.*, 1959, **81**, 2604; J. Meinwald and R. A. Chapman, *ibid.*, 1968, **90**, 3218; W. C. Agosta and D. K. Herron, *ibid.*, p. 7025.
[33] T. Sato and H. Obase, *Tetrahedron Letters*, 1967, 1633.
[34] M. Nazir, Naeemuddin, I. Ahmed, M. K. Bhatty, and Karimullah, *Pakistan J. Sci. Ind. Res.*, 1967, **10**, 13.
[35] C. Y. Meyers, A. M. Malte, and W. S. Mathews, *J. Amer. Chem. Soc.*, 1969, **91**, 7510.
[36] B. Achilladelis and J. R. Hanson, *Phytochem.*, 1968, **7**, 1317.
[37] Reviewed by A. R. Battersby, *Chem. Soc. Specialist Periodical Reports, The Alkaloids*, 1971, **1**, 31, and references therein.
[38] P. G. Strange, J. Staunton, H. R. Wiltshire, A. R. Battersby, K. R. Hanson, and E. A. Havir, *J.C.S. Perkin I*, 1972, 2364.

(10 ml) at 5° Further portions of the reducing agent (0·5 ml) were added hourly, and after 4 h the mixture was cooled to −50° and treated successively with *ca.* 0·3 ml each of methyl acetate, water, and finally saturated aqueous ammonium chloride. The mixture was warmed to 20°, diluted with water (50 ml), and extracted with ether; the combined extracts were washed with saturated brine, dried, and evaporated to yield [2-$^{14}$C]geraniol (0·78 mCi). G.l.c. showed 90% of geraniol with less than 4 and 6%, respectively, of citronellol and nerol.

*Administration of Labelled Precursors.*—The foregoing product (0·39 mCi) was dispersed in 1% aqueous Tween 80 solution (20 ml) containing adenosine triphosphate[39] (ATP) (0·1 mg ml$^{-1}$) and the clear solution was divided equally among 40 glass test tubes (1·2 × 0·75 cm). A vigorous shoot (*ca.* 3 in long) was cut in June from a specimen of *Salvis officinalis*, the cut end was immediately immersed in distilled water and, after 1 min, the lower 0·5 cm of the shoot was cut off and rejected. The cut end of the shoot was then transferred rapidly into the solution in the feeding tube. When the shoot had absorbed almost all of the solution, the remainder was ' washed in ' with aqueous ATP (0·1 mg ml$^{-1}$; 2 × 0·1 ml) and the shoot was then transferred to aqueous ATP (10 ml) until harvested 29 h later; 100 shoots were treated in this way. The feeding tubes finally contained 8% of the original $^{14}$C activity.

The extraction of (−)-camphor and (−)-borneol from the shoots was carried out as under ' Preparative Separation of Terpenes ' save that radio-inactive (−)-camphor (155 mg) and (−)-borneol (153 mg) were added with the ground plant material to the Soxhlet thimble; yields of fractions from preparative g.l.c.: (−)-camphor 43 mg, (−)-borneol 130 mg.

Part of the borneol (27 mg) in light petroleum (b.p. 100—120°) was treated at 20° with phenyl isocyanate (0·16 g). The flask was sealed and kept at 20° for 8 h, then at 0° for 48 h, and finally the suspension was heated to 80—90°. Filtration removed the insoluble diphenylurea and the bornyl phenylurethane crystallised from the filtrate; it was recrystallised from light petroleum to constant specific activity (Table 3); m.p. 136° (Found for radio-inactive material: C, 74·9; H, 8·2; N, 5·1. Calc. for $C_{17}H_{22}NO_2$: C, 74·7; H, 8·5; N, 5·1%).

Half of the (−)-[$^{14}$C]camphor (21·5 mg) was mixed in ethanol (4 ml) with radio-inactive (−)-camphor (180 mg), and hydroxylamine hydrochloride (0·2 g) and pyridine (0·3 ml) were added. After the solution had been heated under reflux for 3 h, g.l.c. showed that *ca.* 1% of the original camphor remained. The residue from evaporation of the ethanol was washed with water (2 × 2 ml) and dried, and the oxime was dissolved in ether (filtration removed traces of hydroxylamine hydrochloride). Evaporation of the filtrate and recrystallisation of the residue to constant specific activity (Table 3) from aqueous ethanol gave (−)-camphor oxime, m.p. 118—120° (Found for radio-inactive material: C, 71·6; H, 10·1; N, 8·3. Calc. for $C_{10}H_{17}NO$: C, 71·8; H, 10·2; N, 8·4%).

The various exploratory tracer experiments on other plants (see main text) were carried out in a similar way. For *Artemesia californica*, 123 shoots were used (20—30 cm long; fresh wt. 98 g) and [2-$^{14}$C]geraniol (0·39 mCi) was administered to them in July as before. The terpenes were isolated after 45 h as for *S. officinalis* but with the addition of radio-inactive (+)-camphor (274 mg) and (+)-bornyl acetate (216 mg) as carriers to yield (+)-camphor (423 mg) and (+)-bornyl acetate (145 mg) by preparative g.l.c. The oxime of the former was virtually radio-inactive after three recrystallisations (400 counts per 100 s mmol$^{-1}$).

All the foregoing (+)-bornyl acetate, diluted with inactive (+)-bornyl acetate (239 mg), was dissolved in light petroleum (5 ml) and stirred vigorously with aqueous N-sodium hydroxide for 4 h; complete hydrolysis to (+)-borneol occurred (g.l.c.). The borneol (270 mg) was oxidised as described later to (+)-camphor, which was purified by preparative g.l.c. (yield 92 mg) and then converted as earlier into (+)-camphor oxime. Recrystallisation thrice from aqueous ethanol gave radio-inactive oxime.

The inactive (+)-bornyl acetate used was prepared by reduction of (+)-camphor with lithium aluminium hydride–aluminium chloride,[40] chromatography of the products,[41] and acetylation of the (+)-borneol so obtained with acetic anhydride and pyridine. The product was purified by preparative g.l.c.

*Dehydration of 2-Phenylborneol*[28] (13).—(a) *On alumina.* The alcohol (0·2 g) was transferred in benzene to a column of Woelm neutral alumina (activity I), and after 30 min the column was eluted with benzene to give a mixture (*ca.* 40 : 60) of 2-phenylbornene (14) and 2-phenylcamphene (15) (t.l.c. and n.m.r. analysis).

(b) *In hot dimethyl sulphoxide.* A solution of 2-phenylborneol (0·2 g) in dimethyl sulphoxide (0·6 g) was heated at 160—180° for 18 h, then cooled, diluted with water (20 ml), and extracted with light petroleum (b.p. 40—60°). The extracted material was almost identical with the mixture from methanol (a).

(c) *In hot dimethylformamide.* Part of the alcohol (0·2 g) was unchanged after being heated at 130—140° in dimethylformamide (1 ml) for 96 h; the hydrocarbon fraction contained mainly the olefin (14), but (15) was also present (t.l.c.).

*Degradation of (−)-Camphor.*—(a) *Rearrangement.* Acetyl chloride (8 ml) was added at 0° during 10 min to (−)-camphor oxime (6·4 g), and the mixture was stirred as it warmed to room temperature. The temperature of the mixture then rose spontaneously to 80°; when it had fallen again the product was poured into ice–water (100 g) and extracted with benzene. The extracts were washed with saturated sodium hydrogen carbonate solution, then with saturated brine, dried, and evaporated. Chromatography of the residue in n-pentane on Woelm neutral alumina (activity I; 45 g) gave α-campholenic nitrile (2,2,3-trimethylcyclopent-3-enylacetonitrile) (3·85 g), homogeneous by g.l.c.; $\nu_{max.}$ 2240 and 1650 cm$^{-1}$; τ 9·2 (3H, s), 8·98 (3H, s), 8·42 (3H, d), 7·78 (*ca.* 4H, m), and 4·78 (1H, m) (*cf.* ref. 33).

The conditions were modified for (−)-[$^{14}$C]camphor oxime (160 mg; 4·8 × 10$^3$ disint. per 100 s mmol$^{-1}$) because of the small scale. After addition of acetyl chloride (0·21 ml) at 20°, the mixture was heated at 35° for 10 min and was then worked up as before to give radioactive α-campholenic nitrile in n-pentane. This solution was concentrated ready for direct reaction with phenylmagnesium bromide.

(b) 2,2,3-*Trimethylcyclopent-3-enylmethyl phenyl ketone* (21). α-Campholenic nitrile (2·3 g) in ether (25 ml) was added during 15 min to a boiling solution of phenylmagnesium bromide [from magnesium (0·6 g) and bromobenzene

[39] D. V. Banthorpe and K. W. Turnbull, *Chem. Comm.*, 1966, 177.

[40] E. L. Eliel and D. Nasipuri, *J. Org. Chem.*, 1965, **30**, 3809.

[41] B. Gastambide, *Ann. Chim.* (*France*), 1954, **9**, 257.

(4 g)] in ether (35 ml). The mixture was heated under reflux for 4·5 h, cooled to 5°, and treated in portions with aqueous ammonium chloride (2 g in 6 ml). After the ether had been evaporated, the mixture was stirred at 50—60° for 1 h, then at 20° for 16 h, and finally was extracted with ether. The oil recovered from the ether was fractionated on alumina, first in n-pentane and then with 1 : 1 n-pentane–ether to give 2,2,3-*trimethylcyclopent*-3-*enylmethyl phenyl ketone* (1·98 g). Final purification was achieved by preparative g.l.c. [6 × $\frac{3}{8}$ in column of Carbowax 20M (20% w/w) on Chromosorb P (60—80 mesh); *T* 210°]; *m/e* 228 ($M^+$), 108, 105, 93, and 77, *m** 80 (108 ⟶ 93); $\nu_{max.}$ 1685 $cm^{-1}$; τ 9·17 (3H, s), 9·0 (3H, s), 8·4 (3H, d), 7·7 and 7·1 (*ca.* 5H, centres of m), 4·78 (1H, m), and 2·55 and 2·1 (5H, centres of m, aromatics).

The foregoing ketone was converted into its *oxime* essentially as for the preparation of camphor oxime; m.p. 117—118° (from ethanol) (Found: C, 79·1; H, 8·7; N, 5·6. $C_{16}H_{21}NO$ requires C, 79·0; H, 8·7; N, 5·8%).

Reaction in the $^{14}C$-series was carried out similarly by adding the nitrile in n-pentane plus ether (1·5 ml) to phenylmagnesium bromide [from magnesium (37·5 mg) and bromobenzene (0·26 g)].

(c) *Cleavage of the ketone* (21). A solution of all the foregoing [$^{14}C$]ketone in t-butyl alcohol (0·5 ml), carbon tetrachloride (1 ml), and water (0·1 ml) was stirred vigorously at 5° as powdered potassium hydroxide (0·8 g) was added in one portion, and stirring was continued for 5 min at 5°. The mixture was then placed in a preheated oil-bath at 52°, stirred for 30 min, cooled, and extracted four times with ether. The aqueous phase was acidified at 0° with 6N-hydrochloric acid and extracted four times with ether. After the combined extracts had been washed with saturated brine, they were dried and evaporated. Extraction of the residue with hot water gave benzoic acid, which was recrystallised from water to constant specific activity of 4·6 × $10^3$ disint. per 100 s $mmol^{-1}$.

*Degradation of* (−)-*Borneol.*—The remaining (−)-[$^{14}C$]-borneol (103 mg) was diluted (to 250 mg) and then was dissolved in glacial acetic acid (0·19 ml). To this stirred solution was added, during 8 h, a solution of chromium trioxide (0·45 g) in water (0·26 ml) and glacial acetic acid (0·45 ml). After being stirred for a further 36 h, the mixture was diluted with water (4 ml) and extracted with ether, and the combined extracts were washed with water and saturated aqueous sodium hydrogen carbonate. Most of the ether was removed at low temperature and the residue was fractionated by preparative g.l.c. to give (−)-camphor (70 mg; 28%). Trial runs on inactive material gave >60% yields of camphor before g.l.c.; the subsequent loss is due to incomplete trapping from the gas stream.

The (−)-[$^{14}C$]camphor was converted as earlier into its oxime and diluted with inactive (−)-camphor oxime (124 mg) before recrystallisation from aqueous ethanol to constant specific activity of 3·4 × $10^3$ disint. per 100 s $mmol^{-1}$. This was converted by way of the nitrile (20) into the ketone (21), which was cleaved as before to yield benzoic acid; after four crystallisations this reached constant activity of 2·8 × $10^3$ disint. per 100 s $mmol^{-1}$.

We thank Messrs. Bush, Boake, Allen Ltd. for gifts of monoterpenes and the S.R.C. for financial support. We also thank Dr. D. V. Banthorpe (University College, London) for exchange of information and Mr. J. K. Hulme (Ness Botanic Garden) and Mr. J. Simons (University Botanic Garden, Cambridge) for provision of plant materials.

[2/1403 *Received*, 16*th June*, 1972]