# The Synthesis of (1*R*)-[2-$^{18}$O]-α-Fenchocamphoronequinone. Specific Labeling of One Carbonyl Group in a Norbornane-2,3-dione[1]

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*Received February 20, 1973*

A method has been devised for the preparation of a norbornane-2,3-dione with one of the carbonyl groups enriched specifically in $^{18}$O, *viz.*, oxidation of a labeled ketone with selenium dioxide in acetic anhydride. This method has been applied to the oxidation of labeled, optically active α-fenchocamphorone giving specifically labeled α-fenchocamphoronequinone. This diketone, whose optical activity is due only to $^{18}$O substitution, showed a small but measureable effect in the CD of both low intensity absorption bands in the region of 250–520 nm.

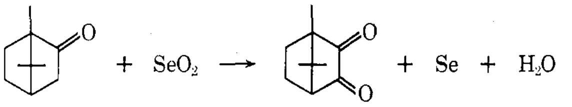
Compounds which derive optical activity from isotopic substitution offer interesting possibilities for studying the origin of vibronic absorption bands if these bands are accessible to CD measurements. Up to now much work has been done to synthesize compounds whose optical activity stems from deuterium substitution.[2] In addition a few examples have been reported where optical activity is due to oxygen isotopes, *viz.*, some $^{16}$O–$^{18}$O sulfones[3,4] and $^{16}$O–$^{18}$O sulfonate esters.[3b] Measurement of the optical activity in these compounds has usually resulted in plain ORD curves.[5]

In view of this situation it seemed worthwhile to start a program for the synthesis of ketones and diketones with optical activity due to isotopic substitution. α diketones were particularly inviting because two low intensity absorption bands in the region of 250–520 nm can be studied by CD provided that the effect is large enough.

One of our efforts was directed toward the synthesis of a specifically labeled $^{16}$O–$^{18}$O α diketone starting from optically active norcamphor or α-fenchocamphorone (**5**). Because of the availability and the price of starting materials ($H_2{}^{18}O$, *e.g.,*) it was decided to try out the various steps in the synthesis with cheaper materials. This resulted in a number of interesting observations which will be discussed first.

**Model Experiments.**—Initially the synthesis of specifically labeled camphorquinone was attempted from camphor. Water, enriched in $^{18}$O (2.095 at. % $^{18}$O[6]), was used for labeling the reagents, since an $^{18}$O label of 2% is sufficiently high to detect the possible exchange of oxygen between the reagents and the reaction medium by mass spectroscopy.

An α diketone is most conveniently prepared by the oxidation of a ketone with selenium dioxide.[7] Because the water formed in this reaction (eq 1) reacts with selenium dioxide to form selenious acid, which might catalyze in unfavorable conditions exchange of oxygen between the carbonyl group and water,[8] a solvent was required in which either selenious acid was insoluble or the water formed could be removed.



(1)

In a first experiment labeled camphor was oxidized with selenium dioxide in acetic anhydride[9] (molar ratio of camphor, selenium dioxide, and acetic anhydride, 0.33:0.54:0.53), the intention being to bind the water formed with acetic anhydride. This first experiment was a failure: the camphorquinone prepared from labeled camphor (2.26% $^{18}$O) had too low a label (1.62% $^{18}$O; retention is 2.45% $^{18}$O).

Various possible reasons for this loss of label were systematically investigated. First it was verified that labeled camphor does not lose label[10] when boiled with acetic anhydride for 4 hr.[11,12] Similarly no loss of label occurred when 0.3 g of water was added to a boiling solution of 2.5 g of labeled camphor in 2.5 ml of acetic anhydride[13] (molar ratio of camphor, water, and acetic anhydride, 0.33:0.33:0.53). Apparently under these conditions the reaction between water and acetic anhydride is much faster than the acetic acid catalyzed exchange reaction between water and camphor.

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[1] Taken from the thesis of the author, Leiden (1973).

[2] For references on hydrogen–deuterium asymmetry, see D. Arigoni and E. L. Eliel, *Top. Stereochem.*, **4**, 127 (1969); L. Verbit, *Progr. Phys. Org. Chem.*, **7**, 51 (1970).

[3] (a) C. J. M. Stirling, *J. Chem. Soc.*, 5741 (1963); (b) M. A. Sabol and K. K. Andersen, *J. Amer. Chem. Soc.*, **91**, 3603 (1969).

[4] R. Annunziata, M. Cinquini, and C. Colonna, *J. Chem. Soc., Perkin Trans. 1*, 2057 (1972).

[5] Only two exceptions are known (H–D asymmetry). *Cf.* S. Englard, J. S. Britten, and I. Listowsky, *J. Biol. Chem.*, **242**, 2255 (1967); L. Verbit, *J. Amer. Chem. Soc.*, **89**, 167 (1967).

[6] Determined by Miles Laboratories, Inc.

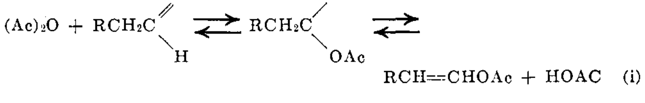
[7] For references to the literature on the application of selenium dioxide in organic chemistry, see the following. L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley-Interscience, New York, N. Y.: Vol. 1, 1967, p 992; Vol. 2, 1969, p 360; Vol. 3, 1972, p 245. C. F. Cullis and A. Fish, "The Chemistry of the Carbonyl Group," S. Patai, Ed., Vol. 1, Interscience, New York, N. Y., 1966, p 159. E. N. Trachtenberg, "Oxidation," R. L. Augustine, Ed., Vol. 1, Marcel Dekker, New York, N. Y., 1969, p 119.

[8] M. Byrn and M. Calvin, *J. Amer. Chem. Soc.*, **88**, 1916 (1966); P. Greenzaid, Z. Luz, and D. Samuel, *Trans. Faraday Soc.*, 2787 (1968).

[9] W. C. Evans, J. M. Ridgion, and J. L. Simonsen, *J. Chem. Soc.*, 137 (1934).

[10] In the low label experiments the maximum absolute error in the determination of $^{18}$O labels was ±0.10%. Therefore a statement such as "retention of label was observed" should read "relative loss of label was 5% or less."

[11] Thus the mechanism responsible for the loss of $^{18}$O when a labeled aldehyde is treated with acetic anhydride (eq i) does not apply here; *cf.*



$$(Ac)_2O + RCH_2C\overset{O}{\underset{H}{\lessgtr}} \rightleftharpoons RCH_2C\overset{OAc}{\underset{OAc}{\lessgtr}} \rightleftharpoons RCH{=}CHOAc + HOAC \quad (i)$$

Houben-Weyl, "Methoden der Organischen Chemie," Georg Thieme Verlag, Stuttgart, Bd 7/1, 1954, p 442.

[12] When camphor is treated with trichloroacetic acid anhydride (110–120°) a geminate diester is formed, 2,2-dihydroxycamphane ditrichloroacetate, which decomposes to give after a Wagner-Meerwein rearrangement 1-hydroxycamphene trichloroacetate as the primary product: J. Libman, M. Sprecher, and Y. Mazur, *Tetrahedron*, **25**, 1679 (1969).

[13] This quantity of water (0.3 g) was chosen because it would have been formed when selenium dioxide had been added to the reaction mixture and had oxidized all of the ketone and nothing else.

Reconsidering in the light of these results the oxidation experiment in which loss of label did occur, it was realized that both ketone and solvent[14] were oxidized by selenium dioxide so that toward the end of the reaction hardly any acetic anhydride was left. The lifetime of a water molecule then became long enough to permit oxygen exchange with the carbonyl group.

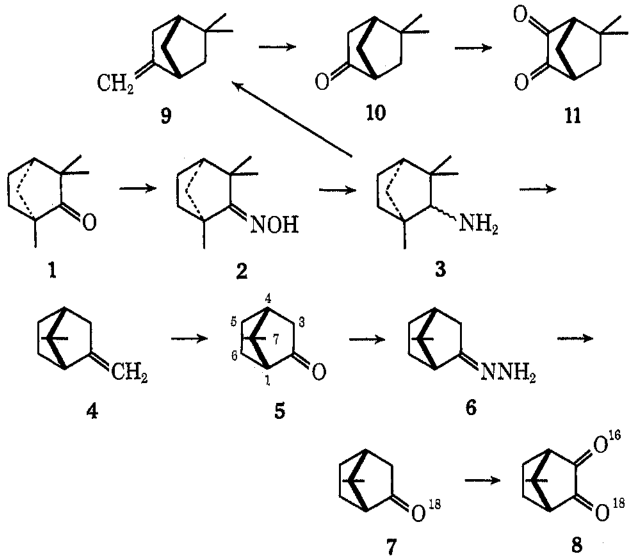
Indeed it was found that loss of $^{18}$O in the oxidation of labeled camphor can be prevented by sufficiently reducing the quantity of selenium dioxide with respect to camphor and acetic anhydride. Although the mass spectra of camphorquinone clearly showed retention of label[10] and that selective label incorporation had been achieved,[15] the possibility of interchange of the two oxygen atoms within one diketone molecule could not be excluded on the basis of these results.[16] On the other hand this process seemed to be very improbable so that continuation of the synthesis with highly labeled materials seemed justified.

Meanwhile two other routes to the desired compounds had been explored. It appeared that the oxidation of labeled camphor with selenium dioxide in toluene[17] proceeds with retention of label, although in poor yield. In this case water is probably removed by the excess of unreacted selenium dioxide.

Finally it was tried to devise an oxidation reaction for the preparation of camphorquinone where water could not possibly be a reaction product, so that trouble due to exchange with water could not occur. The oxidation of camphor enol benzoate was attempted with selenium dioxide in benzene. Camphorquinone and benzoic acid were formed in good yield. However, when labeled selenium dioxide[18] was employed, randomly labeled camphorquinone was obtained.

**Experiments with High Label Incorporation.**—The route followed to (1*R*)-[2-$^{18}$O]-α-fenchocamphoronequinone (**8**) is indicated in Scheme I (absolute configurations are depicted[19]).

SCHEME I
THE SYNTHESIS OF THE TITLE COMPOUND FROM (+)-(1*S*)-FENCHONE



The required α-fenchocamphorone (**5**) was prepared *via* α-fenchene (**4**) from fenchone (**1**); this route was chosen because it was judged to be the one by which α-fenchene (**4**) of the highest chemical purity could be obtained.[20] The α-fenchocamphorone (**5**) thus obtained did not contain the most likely impurity β-fenchocamphorone (**10**), because after selenium dioxide oxidation the diketone obtained was optically inactive (no effect in CD), *i.e.*, the diketone did not contain a measurable quantity of β-fenchocamphoronequinone (**11**) as an impurity.[21]

α-Fenchocamphorone (**5**) was labeled by regeneration from the hydrazone (**6**) with water enriched in $^{18}$O. This labeled ketone contained 62.72% $^{18}$O. After oxidation with selenium dioxide in acetic anhydride some unreacted ketone was recovered with a label of 60.19% $^{18}$O. Of the diketone prepared 48.05% was specifically labeled with $^{18}$O, and 0.08% was doubly labeled. Completely specific labeling had been achieved.[22]

Much less label is missing from the ketone recovered than from the diketone formed. One might suppose that oxygen exchange of the diketone with water formed during the selenium dioxide oxidation is faster than oxygen exchange of the starting monoketone. The results of following two exchange experiments confirmed this hypothesis.

A homogeneous solution of α-fenchocamphorone (0.5754 g), α-fenchocamphoronequinone (0.5063 g), labeled water (1.210 g, 12.062 at. % $^{18}$O[6]), and 0.20 *N* acetic acid in dioxane (1.1230 g) was left at room temperature for 96 hr. After work-up it was shown that the ketone had hardly exchanged oxygen (label 0.38% $^{18}$O; no label is 0.2% $^{18}$O), whereas 14.90% of the diketone was labeled with one $^{18}$O.

Labeled α-fenchocamphorone and α-fenchocamphoronequinone were dissolved in acetic anhydride and to the boiling solution the quantity of water calculated to hydrolyze the anhydride was gradually added. Both

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[14] J. J. Postowsky and B. P. Lugowkin, *Ber.*, **68**, 854 (1935); L. Rappen, *J. Prakt. Chem.*, *n.F.*, **157**, 196 (1941).

[15] A sample of camphorquinone prepared from labeled camphor[9] had a label of 1.50% $^{18}$O and the fragment (M − CO) a label of 0.31% $^{18}$O (15-eV spectrum). If both carbonyl groups were equivalent, or if we had a randomly labeled diketone, we should expect the fragment (M − CO) to have a label of 0.75% $^{18}$O.

[16] Assuming that the "chain branching rule" (*cf.* F. W. McLafferty, "Interpretation of Mass Spectra," W. A. Benjamin, New York, N. Y., 1967, p 82) can be used to predict that in camphorquinone the bond between C-1 and C-2 is broken preferentially to the bond between C-3 and C-4, we conclude from the labels of footnote 15 that [2-$^{18}$O]camphorquinone is the main or possibly the only reaction product. If preference for bond rupture should be opposite to the "chain branching rule," then [3-$^{18}$O]-camphorquinone is the main or the only reaction product, and interchange of oxygen atoms within a molecule has taken place.

[17] J. Vène, *C. R. Acad. Sci.*, **216**, 772 (1943).

[18] Labeled selenium dioxide is commercially available (Miles Laboratories, Inc.).

[19] The absolute configurations follow from the absolute configuration of camphor [M. G. Northolt and J. H. Palm, *Recl. Trav. Chim. Pays-Bas*, **85**, 143 (1966)] and the relative configurations of fenchone (**1**) and camphor [A. Fredga and J. K. Miettinen, *Acta Chem. Scand.*, **1**, 371 (1947)].

[20] For syntheses of α-fenchene (**4**) the reader is referred to (a) J. L. Simonsen, "The Terpenes," Vol. 2, 2nd ed, Cambridge University Press, New York, N. Y., 1949, p 538; (b) E. Gildemeister and Fr. Hoffmann, "Die Aetherischen Oele," Akademie-Verlag, Berlin, 4, Aufl., Bd IIIa, 1960, p 197; (c) V. Mattinen, *Ann. Acad. Sci. Fenn.*, *Ser. AII*, **105**, 22 (1961); *Chem. Abstr.*, **56**, 4798*i* (1962).

[21] Unfortunately the CD of β-fenchocamphoronequinone (**11**) has not been published, but, if we assume that its strongest CD band in the region of 250–520 nm has Δε 0.1, which is a rather low value, then we would have detected **11** by CD if its concentration in α-fenchocamphoronequinone had been >0.35%.

[22] The presence of doubly labeled diketone (0.08%) is due to the natural abundance of $^{18}$O in the oxidizing agent.

ketone and diketone lost label, but the diketone much faster than the ketone.[23]

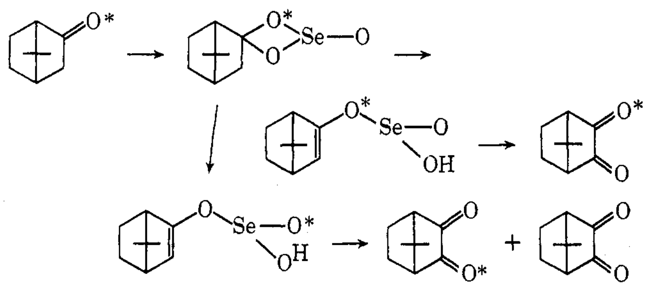
**Optical Purity.**—Our starting fenchone oxime (**2**) had $[\alpha]_D$ +41.19° (absolute EtOH); comparison with a reliable value of $[\alpha]_D$ +46.5° (EtOH)[29] indicates an optical purity at 88.6%.

The physical constants recorded by Rassat[30] for (1*S*)-α-fenchocamphorone (the antipode of **5**), $[\alpha]_D$ −60° (EtOH), $\Delta\epsilon_{max}$ −1.60 (cyclohexane), are not consistent with our best data: $[\alpha]_D$ +67.05° (MeOH),[31] $\Delta\epsilon_{max}$ +2.30 (cyclohexane). Mattinen[20c] recorded $[\alpha]_D$ +73.94° (EtOH); if this is taken as the correct value, then our α-fenchocamphorone has an optical purity of 90.7%.

A discussion of the optical purity of the $^{16}$O–$^{18}$O diketone prepared according to Scheme I must involve the mechanism of the oxidation reaction with selenium dioxide. Corey and Schaefer[32] have postulated a selenite enol ester as a reaction intermediate. If such a species is formed *via* a cycloaddition of selenium dioxide to the ketone, then one would expect (Scheme II) in our case 66.67% racemization and 25% loss of label. This mechanism seems unlikely because labeled camphor can be oxidized with retention of label,[10] and our exchange experiments suggest that during the oxidation of α-fenchocamphorane loss of label is due to exchange with water.

Scheme II

A Mechanism Which Gives Rise to Specifically Labeled Diketone, but with Partial Racemization



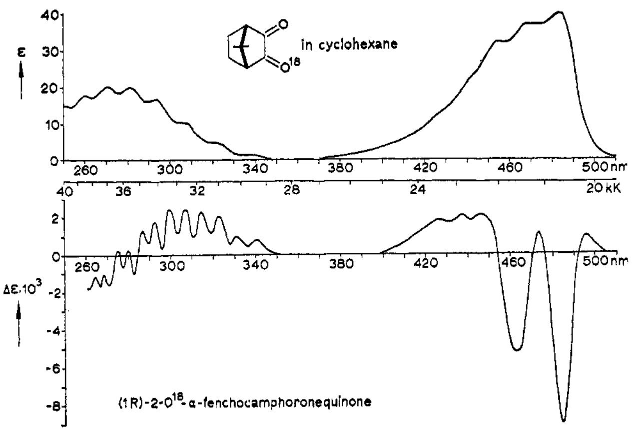


Figure 1.

If we assume that oxidation of labeled α-fenchocamphorone with selenium dioxide gives rise to no racemization because of the mechanism of the oxidation reaction,[33] then (1*R*)-[2-$^{18}$O]-α-fenchocamphoronequinone (**8**) has an optical purity of 90.7%.

Measurement of the CD of specifically labeled α-fenchocamphoranequinone, although very small, proved to be possible. It is displayed in Figure 1 together with the absorption spectrum. These spectra have been published before.[34,35] The influence of $^{18}$O substitution (label 62.72% $^{18}$O) on the CD of α-fenchocamphorone was only small. In Figure 2 the ratio of A/B of the values of Δε in the two maxima would be 1% lower if the CD of the labeled ketone was depicted here instead of the CD of the unlabeled ketone. The dotted line in Figure 2 encloses a part of the graph which is different in the case of the labeled ketone. In Figure 3 this detail of the CD curves of both labeled and unlabeled ketone is enlarged. The influence of

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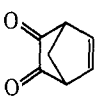
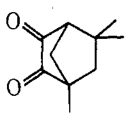
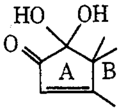
[23] In this context it may be of interest to mention some data found in the literature. The yellow compound dehydronorcamphorquinone (**12**)



**12**



**13**



**14**

gives a colorless solution in water. A hydrate is postulated.[24] Thus this diketone might undergo uncatalyzed oxygen exchange with water, whereas oxygen exchange between water and a ketone requires catalysis.[25] Rassat[26] states that isofenchonequinone (**13**) is hydrated easily. Some norsteroids with an α-diketone chromophore in the unsaturated A ring (**14**) can be isolated as monohydrates.[27] Cyclobutane-1,2-dione even reacts with water to give α-hydroxycyclopropenecarboxylic acid.[28]

[24] H.-D. Scharf, W. Droste, and R. Liebig, *Angew. Chem.*, **80**, 195 (1968).

[25] This statement does not hold at elevated temperatures; *e.g.*, cyclopentanone recovered after heating in a sealed tube (2 hr; 150°); cyclopentanone (1 ml), THF (1 ml), and labeled water (0.5 g, 12.062 at. % $^{18}$O[6]) had a label of 7.36% $^{18}$O. For a similar though less convincing experiment, see M. Cohn and H. C. Urey, *J. Amer. Chem. Soc.*, **60**, 679 (1938).

[26] H.-P. Gervais and A. Rassat, *Bull. Soc. Chim. Fr.*, 743 (1961).

[27] T. Kubota and F. Hayashi, *Tetrahedron*, **23**, 999 (1967).

[28] J.-M. Conia and J. M. Denis, *Tetrahedron Lett.*, 2845 (1971).

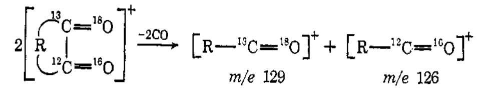
[29] W. Hückel and M. Sachs, *Justus Liebigs Ann. Chem.*, **498**, 166 (1932).

[30] C. Coulombeau and A. Rassat, *Bull. Soc. Chim. Fr.*, 3752 (1966).

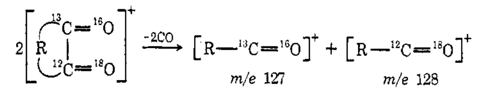
[31] The angles of rotation of α-fenchocamphorone in MeOH and EtOH are identical within the experimental error.

[32] E. J. Corey and J. P. Schaefer, *J. Amer. Chem. Soc.*, **82**, 918 (1960); J. P. Schaefer, *ibid.*, **84**, 713, 717 (1962).

[33] It is possible to prove rigorously that oxidation of **7** is not accompanied by racemization. To prove this **7** has to be prepared, enriched in $^{13}$C in the 2 or 3 position. This [$^{13}$C–$^{18}$O]-**7** has to be oxidized to give **8**. Use has to be made of fragmentation reactions in the mass spectrograph: molecular ions of norcamphorquinones lose CO and OCCO very easily. We can exclude racemization if (1) the fragment (M − 56) is not enriched in $^{13}$C, (2) it follows from measurement of M that **8** is labeled specifically, (3) it is proved by measurement of (M − CO) that oxidation of [2-$^{13}$C–2-$^{18}$O]-**7**, *e.g.*, gives rise exclusively to [2-$^{13}$C–2-$^{18}$O]-**8**. This is possible because scrambling of $^{18}$O (equal to inversion of the absolute configuration if the molecule was not enriched in $^{13}$C) means formation of [2-$^{13}$C–3-$^{18}$O]-**8** which has a structure of the multiplet (M − CO) different from that of [2-$^{13}$C–2-$^{18}$O]-**8**; *cf.* eq ii and iii [a fragmentation reaction of **8** labeled with



$$2\left[R\begin{matrix}{}^{13}C{=}{}^{18}O\\ |\\ {}^{12}C{=}{}^{16}O\end{matrix}\right]^{+} \xrightarrow{-2CO} \underset{m/e\ 129}{[R{-}{}^{13}C{=}{}^{18}O]^{+}} + \underset{m/e\ 126}{[R{-}{}^{12}C{=}{}^{16}O]^{+}} \qquad \text{(ii)}$$



$$2\left[R\begin{matrix}{}^{13}C{=}{}^{16}O\\ |\\ {}^{12}C{=}{}^{18}O\end{matrix}\right]^{+} \xrightarrow{-2CO} \underset{m/e\ 127}{[R{-}{}^{13}C{=}{}^{16}O]^{+}} + \underset{m/e\ 128}{[R{-}{}^{12}C{=}{}^{18}O]^{+}} \qquad \text{(iii)}$$

$^{13}$C and $^{18}$O in the chromophore (R = $C_7H_{12}$)]. 2-$^{13}$C–2-$^{18}$O-**8** gives in the fragment (M − CO) a relative increase of intensity of the peak for *m/e* 129 compared with unlabeled **8** (eq ii). 2-$^{13}$C–3-$^{18}$O-**8** gives in the fragment (M − CO) a relative increase of intensity of the peaks for *m/e* 127 and 128 compared with unlabeled **8** (eq iii).

[34] W. C. M. C. Kokke and L. J. Oosterhoff, *J. Amer. Chem. Soc.*, **94**, 7583 (1972).

[35] The factor used to correct the observed CD for optical and isotopic impurity was 10000/(48.05 × 90.7); 90.7 stands for the optical purity of the $^{16}$O–$^{18}$O diketone and 48.05 for its isotopic purity, *i.e.*, the percentage of molecules labeled with one $^{18}$O.

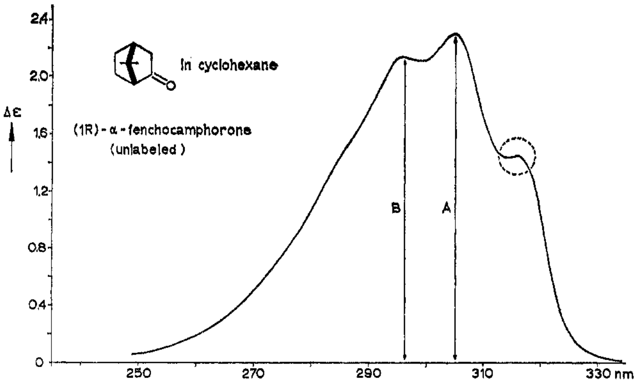


Figure 2.—CD of compound **5**.

$^{18}$O substitution on the absorption spectra of ketone (**7**) and diketone (**8**) could not be detected with a Cary 14 or a Cary 15.

## Experimental Section

Melting points are not corrected. Angles of rotation were determined with a Bendix-NPL photoelectric polarimeter at room temperature.

**Mass spectra.**—Labels were calculated from peak intensities in spectra obtained with a MS-9 mass spectrometer. Because of the various methods to calculate percentage labeling from peak intensities, we give a numerical example of the method we have used. In the mass spectrum of a sample of labeled camphorquinone ($C_{10}H_{14}O_2$) peaks due to molecular ions are at $M/e$ 166, 167, and 168 with relative intensities of 100, 11.19, and 2.275. Correction of the peak intensities for $M/e$ 167 and 168 for satellites of $M/e$ 166 due to D and $^{13}$C is done by comparison with a mass spectrum of $C_{10}H_{14}$. Relative peak intensities for $M/e$ 134, 135, and 136 in $C_{10}H_{14}$ are 100, 11.03 and 0.55.[36] The $^{18}$O label is equal to $[(2.275 - 0.55)/[100 + (11.19 - 11.03) + (2.275 - 0.55)] \times 100\% = 1.67\%$ $^{18}$O. Note, the *absolute* $^{18}$O content is calculated; no correction for natural abundance is applied.

**Camphor Hydrazone**[37] was recrystallized from isooctane. Stored at −20° over $P_2O_5$ it did not liquefy as observed in by Reusch, *et al.*,[37] and after a year the crystals had only turned slightly yellowish.

**Camphor enol benzoate,** prepared according to Lees,[38] appeared to be very impure (glpc). The composition of the reaction product depends on the reaction time: when camphor is refluxed with benzoyl chloride during 4 hr, the enol ester is the main reaction product, but when refluxed overnight another component of the mixture (probably the benzoate of 1-hydroxycamphene) becomes the main product. The enol ester was purified by column chromatography over silica gel. Elution with carbon tetrachloride then gave pure camphor enol benzoate. The enol ester is a liquid at room temperature. Nmr data ($CCl_4$) (shifts with respect to TMS) include three methyl groups at $\delta$ 0.790, 0.986, 1.042 ppm; a triplet at $\delta$ 2.831 ppm [$J = 2 \times 3.50$ Hz; H attached to $C_4$ (bridgehead proton)]; and a doublet at $\delta$ 5.742 ppm [$J = 3.72$ Hz; H attached to $C_3$ (vinylic proton)].

**Labeling of Camphor by Hydrolysis of Camphor Hydrazone.**—A mixture of camphor hydrazone (8.3 g), labeled water (4.0 ml, 2.095 at. % $^{18}$O$^{6}$), and ethylene chloride (50 ml) was placed in a heavy-walled, long-necked flask at a high vacuum line and degassed, and hydrogen bromide [1.67 l./20° (1 atm)] was then frozen into it. The sealed mixture was left overnight, then heated whilst magnetically stirring for 8 hr at 80°. Normal isolation procedures then gave the labeled camphor which was purified twice by sublimation to give 6.0 g of camphor, label 2.26% $^{18}$O.

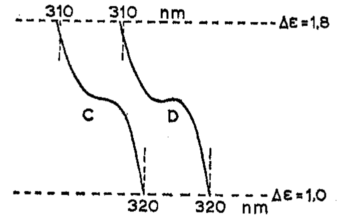


Figure 3.—Detail of the CD of labeled (C) and unlabeled (D) α-fenchocamphorone (in cyclohexane).

**Labeling of Selenium Dioxide by Exchange.**—Highly labeled selenium dioxide was prepared by exchange between selenium dioxide (4.9 g) and deuterated water (1.0 g, 91.8% $^{18}$O; a gift of Professor E. Heilbronner, Basel) (16 hr on a bath at 130°). The water was then removed with a rotatory evaporator until the residue crystallized. Drying was effected in an oven over $P_2O_5$ *in vacuo*. Label was calculated on the basis of complete exchange 31.25% $^{18}$O.

**Oxidations of Labeled Camphor. Two Selected Experiments (A and B).** A.—A mixture of labeled camphor (3.0 g, 2.19% $^{18}$O), selenium dioxide (1.5 g), and acetic anhydride (3 ml) was heated for 3 hr at 145°. After removal of the solvent and sublimation the crude product (1.8 g) was separated by preparative glpc to give camphor (0.8 g) and camphorquinone (0.5 g, label 2.27% $^{18}$O). Retention of label was 2.38% $^{18}$O.

B.—A mixture of labeled camphor (2.5 g, 2.19% $^{18}$O), selenium dioxide (1.85 g), and dry toluene (5 ml) was refluxed for 15.5 hr, then the solvent was removed, and the residue sublimed. Separation of the crude mixture (2.0 g) gave camphor (1.4 g) and camphorquinone (0.1 g) (label 2.32% $^{18}$O). Retention of label was 2.38% $^{18}$O.

**Oxidation of Camphor Enol Benzoate with Labeled Selenium Dioxide.**—A stirred mixture of benzene (9 ml), labeled selenium dioxide (3.0 g, 31.25% $^{18}$O), and camphor enol benzoate (4.4 g) was heated at 150–160° in an autoclave for 3.3 hr. The reaction can be carried out in xylene as well (4 hr, reflux) but we chose benzene because it can be removed more easily. Methylene chloride was then added to the cooled reaction mixture which was filtered. Benzoic acid was removed by washing with $NaHCO_3$ solution. After sublimation the camphorquinone was purified by recrystallization (twice) from cyclohexane; the mother liquors were worked up by preparative glpc (SE-30 column). The yield was 2.0 g; 30.95% of the molecules was labeled with one $^{18}$O; 3.58% was doubly labeled; the fragment (M − CO) had a label of 19.00% $^{18}$O (32.5-eV spectrum), 19.07% $^{18}$O (15-eV spectrum). These data seem consistent with a diketone, label 19.14% $^{18}$O, the $^{18}$O randomly distributed over the carbonyl groups; *viz.*, expected for this case were 30.95% of the molecules labeled with one $^{18}$O, 3.66% doubly labeled, a fragment (M − CO) with a label of 19.14% $^{18}$O. We should expect a random distribution of $^{18}$O over the carbonyl groups only if both oxygen atoms of the diketone formed were provided by selenium dioxide, but the label of the diketone is about two-thirds of the value which this mechanism would suggest (19.14% instead of 31.25% $^{18}$O); this loss of label might be due to exchange prior to oxidation.

According to our measurements, when optically pure camphor is used for the preparation of the enol benzoate, then oxidation of this enol benzoate gives optically pure camphorquinone.

**(+)-Fenchone oxime (2)** was prepared from (+)-fenchone (Fluka, purum) according to Wallach[39] in at least 80% yield: $[\alpha]_D$ +41.19° (absolute EtOH), mp 162–164° after recrystallization from heptane and dilute alcohol; lit.[28] $[\alpha]_D$ +46.5° (EtOH), mp 167°.

**Fenchylamine (3)** was prepared from **2** by reduction[40] with sodium and alcohol. The hydrochloride after two recrystallizations from dioxane had a specific rotation of $[\alpha]_D$ −4.53° (MeOH).

**(−)-α-Fenchene (4).**—The amine **3** was regenerated from the hydrochloride and treated with nitrous acid.[41] The reaction products were separated by fractional distillation using a Nester-Faust spinning band column (~20 cm). From 1.5 kg of 1 86 g

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[37] W. Reusch, M. W. DiCarlo, and L. Traynor, *J. Org. Chem.*, **26**, 1711 (1961).

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of α-fenchene fractions was obtained (purity >87.4%) that were used for the preparation of α-fenchocamphorone (**5**). For the measurements more pure **4** was obtained by careful redistillation (~20 cm, 2 ml/hour) of a forerun. The purest sample (99.54%) had [α]D −42.62° (ethyl acetate).

**(+)-α-Fenchocamphorone (5).**—Some early terpene chemists[42] have prepared **5** by ozonization of **4**, but Mattinen[20c] failed to reproduce their reasonable yields. We prepared **5** by oxidation of **4** with ruthenium tetroxide in methylene chloride in 65.5% yield. A sample of the crude ketone was sublimed; then mp 91–96°, [α]D +57.93° (MeOH), was found. Another sample was oxidized with selenium dioxide,[9] and after distillation the oxidation product was purified by preparative glpc (SE-30 column) to remove unreacted ketone. After sublimation the α-fenchocamphoronequinone had mp 139.0–139.5°. No effect in CD could be detected. Therefore **5** was not contaminated with β-fenchocamphorone (**10**), because it would have been possible to detect β-fenchocamphoronequinone (**11**) by CD.[21]

**(−)-α-Fenchocamphorone hydrazone (6)** was prepared from **5** in the manner of Reusch, *et al.*,[37] in 74.4–82.2% yield. **6** was a liquid at room temperature, but crystallized on storing at −15°. Different angles of rotation were recorded for two preparations: [α]D −51.27° and −58.32° (MeOH).

**Hydrolysis of 6. Introduction of the Label. Labeling Procedure.**—Into a degassed mixture of **6**, labeled water, and ethylene chloride, attached to a high vacuum line, HBr was introduced. The method used is the same as was pursued for the labeling of camphor by hydrolysis of its hydrazone, but it was found more convenient to introduce HBr in the reaction mixture using a break-seal vessel. A break-seal vessel with two taps and a ground-glass joint (Figure 4) was flushed with HBr, the lower part of the vessel was then immersed in liquid nitrogen, and the calculated quantity of HBr (2 mol of HBr:1 mol of hydrazone) was admitted and condensed. The taps were melted off. Then the vessel was attached to the high vacuum line, the seal broken, the liquid nitrogen removed, and HBr frozen into the reaction mixture. As already described we used 4 g of water for the hydrolysis of 8.3 g of camphor hydrazone, and we obtained 6.0 g of camphor and 2.0 g of residue (azine). It might be expected that further reduction of the quantity of water with respect to the hydrazone would result in an increase of the yield of azine. In the case of **6** azine formation is sterically more easy than in the case of camphor hydrazone. Thus, when 8.3 g of camphor hydrazone was hydrolyzed with 2.0 g of water, 4.4 g of camphor and 3.2 g of residue were obtained, but **6** (7.6 g), hydrolyzed with water (2.0 g) in ethylene chloride (50 ml), gave 4.4 g of residue and 2.65 g of **5**, mp 100–105°, [α]D +65.10° (MeOH).

**Labeling.**—Water containing 0.31 at. % $^{17}$O, 62.88 at. % $^{18}$O, and 64.0 at. % D[6] was used. We used deuterated water enriched in $^{18}$O, because it is less expensive than water enriched in $^{18}$O where the deuterium content has been reduced to natural abundance. Thus **5** will be labeled with $^{18}$O and deuterated, but only in the 3 position. Deuteration of norbornanones is well documented.[43] Introduction of D at the exo-3 position is easy, introduction of a second D is more difficult, and deuteration at the 6 position does not take place under our conditions.

**6** (7.6 g) was hydrolyzed with labeled water (2.0 g) and HBr; **7** (2.45 g) was obtained and 4.75 g of residue of sublimation. Labels were calculated in the assumption that only monodeuteration had occurred: specific 3 deuteration 3.25%, label 62.72% $^{18}$O.

**Specifically Labeled α-Fenchocamphoronequinone. High Label Incorporation.**—**7** (2.40 g, 62.72% $^{18}$O) was heated with selenium dioxide (1.53 g) and acetic anhydride (2.2 ml) (4 hr on a bath at 150°). By use of 20 ml of dry methylene chloride the reaction product was separated from selenium; it was washed with a bicarbonate solution until neutral and then with a saturated NaCl solution. The solvent was removed with suction and the residue distilled. Diketone and unreacted monoketone were separated by preparative glpc (SE-30 column): 0.86 g of **7** was recovered, mp 108–110°, specific 3 deuteration 3.44%, label 60.19% $^{18}$O; 0.55 g of **8** was obtained, 48.05% labeled specifically with $^{18}$O, 50.87% unlabeled, and 0.08% doubly labeled with $^{18}$O. A solution of **8** showed CD in both absorption bands between 250 and 520 nm. That the observed CD in the visible region was due to isotopic substitution could not be called to question because the precursor **7** does not absorb there, but it happens to be that the second absorption band of **8** at ~300 nm coincides with an absorption band of the precursor **7** and thus it had to be made sure that the observed CD of **8** at ~300 nm was indeed due to **8** and not due to the precursor. The CD band which we observed in this region was shaped like the CD band of **7**, but it had a very unusual fine structure. Because of this band shape we suspected that traces of **7** interfered with the CD measurement. Indeed we showed **8** by glpc to be contaminated with 0.1–0.2% of **7**. This impurity was removed by preparative glpc to yield **8** (0.4 g), mp 140.0–140.5°. Then CD was measured again; the observed CD curve is displayed in Figure 1.

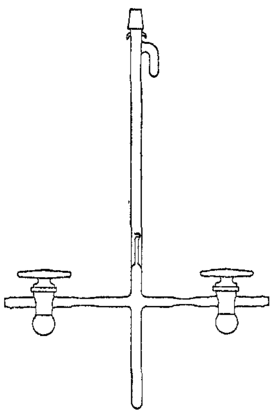

Figure 4.

**The Ultimate Proof That the Observed CD of 8 Is Due Only to Isotopic Substitution Is an Exchange Experiment.**—First CD and absorption were measured of a spectroscopic solution of **8** (24.2 mg) in heptane (10 ml). Then to this solution was added water (4 g) and acetic acid (1 g). This mixture was magnetically stirred at room temperature for 15.25 hr and neutralized, the layers were separated, and the hydrocarbon layer was used for measurement of CD and absorption. No CD could be detected which may serve as a proof of the purity of **8**; the optical density in the visible region decreased by ~50% during this experiment. This might be due to formation of hydrate.[26]

**Data of the CD Curve of 8.**—The following were obtained: uv bands—$\Delta\epsilon_{\max}$ 2.36 × $10^{-3}$ and peaks at 264.5, 268.3, 275.0, 279.8, 286.7, 292.3, 299.3, 306.6, 314.2, 322.5, 331.0, and 340.7 nm; visible band—$\Delta\epsilon_{\min}$ −8.93 × $10^{-3}$ and peaks at 462.5 and 484.5 nm.

**Experiment with Labeled Selenium Dioxide and Unlabeled Ketone.**—Attempted preparation of (1$S$)-2-[$^{18}$O]-α-fenchocamphoronequinone, the antipode of **8**, follows. Using labeled selenium dioxide (31.25% $^{18}$O) and **5**, and conditions as in the experiment with highly labeled **7**, [$^{18}$O]-α-fenchocamphoronequinone was obtained. Some unreacted ketone (label 0.86% $^{18}$O) was recovered. Of the diketone 10.62% was labeled with one $^{18}$O and 0.27% was doubly labeled. If no exchange between selenium dioxide and the reaction medium had occurred, we should expect 31.25% of the diketone to be specifically labeled. Because of the low label the effect in CD of this diketone should be weak, but the two highest peaks in the visible band (Figure 1) should have been above noise level. [The maximum pen deflection expected in CD on the basis of the optical density of the solution of the diketone, its low isotopical purity (10.62%), and the observed CD of **8** (Figure 1) was 9 mm; the noise level of the CD apparatus was 3 mm.] However, no CD could be detected. Presumably (partial) racemization had occurred.

**Exchange Experiment with 7 and 8 (Both Labeled by Exchange) in Acetic Anhydride.**—A solution of **7** (0.4329 g) and **8** (0.4013 g) in acetic anhydride (2.1 g) was heated under reflux. Water was added with a syringe in 5-μl portions at intervals of 3 min. After addition of 30% of the calculated quantity of water (equal to 30% of 0.37 g), a sample of the mixture was taken for the determination of the labels; sampling was also done

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when 50, 70, and 90% of the water had been added. The results are shown in the Table I.

TABLE I

| Water, added, % | Diketone, labeled with one $^{18}O$, % | Ketone, % $^{18}O$ |
|---|---|---|
| 0 | 19.24 | 30.2 |
| 30 | 13.70 | 29.4 |
| 50 | 11.25 | 28.9 |
| 70 | 7.31 | 27.4 |
| 90 | 5.84 | 26.3 |

**Acknowledgment.**—The author is indebted to the Netherlands Organization for the Advancement of Pure Research (Z.W.O) for the sponsoring of this work. A NATO grant for the purchase of $^{18}O$ is gratefully acknowledged. It is with pleasure that the author expresses his gratitude to Dr. N. C. Rol (Shell Research N.V., Amsterdam) and Dr. Klebe, Dr. Van Thuyl, and Mr. Van Houte in this laboratory, who spent a great deal of time on the measurement of $^{18}O$ labels, to Professor W. Parker (Stirling) and to Professor H. Wynberg (Groningen) for their help in improving the presentation of this paper, and to Professor E. Heilbronner (Basel) whose gift of labeled water enabled us to do some experiments with highly labeled selenium dioxide. This work was done in the department of Professor L. J. Oosterhoff.

**Registry No.**—**1**, 4695-62-9; **2**, 40514-83-8; **3**, 21252-46-0; **4**, 7378-37-2; **5**, 40550-41-2; **6**, 40550-42-3; **7**, 40550-43-4; **8**, 40550-44-5; water-$^{18}O$, 14314-42-2; selenium dioxide, 7446-08-4.

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# Chemistry of Cephalosporin Antibiotics. XXVII. 3-Methylenecephams

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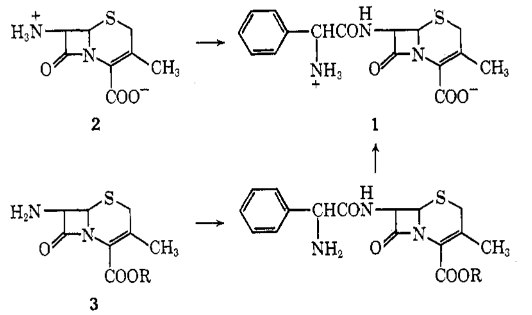
*Recovered March 26, 1973*

Cephalosporanic acids, in which the acetoxy group is displaced by sulfur nucleophiles, were reduced to 3-methylenecephams. Esterification procedures are described for preparing both 3-methylenecepham and 3-methyl-3-cephem esters. 7-Amino-3-methylenecepham-4-carboxylic acid and its esters were isomerized to 7-ADCA and 7-ADCA esters, respectively.

We have had considerable interest in recent years in developing new synthetic routes to deacetoxycephalosporins. A principal member of this series of antibiotics is cephalexin[1] (**1**). Syntheses of this orally active compound include acylations of either 7-aminodeacetoxycephalosporanic acid (7-ADCA, **2**)[2] obtained by hydrogenolysis of 7-ACA[3] or 7-aminodeacetoxycephalosporanic acid esters (**3**)[4] produced in the ring expansion of penicillin sulfoxides.[5] This paper reports the preparation of 3-methylenecephams[6] and their conversions to **2** and **3**.



We anticipated that a selective desulfurization of cephalosporins in which the acetoxy group at $C_3$ methylene has been displaced by sulfur nucleophiles (**4**) would lead to deacetoxycephalosporins (**5**). While some 3-methyl-3-cephems (**5**) indeed formed in the treatment of these sulfur-derivatized cephalosporins with Raney nickel, 3-methylenecephams (**6**) constituted the major products. Other reducing conditions, notably zinc–formic acid–DMF, were also effective in this conversion.

The nmr spectra of 3-methylenecephams show a singlet at $\tau$ 4.9 for the $C_4$ proton and a doublet near $\tau$ 4.7 for the *exo*-methylene grouping. The uv chromophore at 268 m$\mu$, characteristic of $\beta$-lactam-$\Delta^3$-unsaturation system in cephalosporins, is not seen with 3-methylenecephams. 3-Methylenecepham acids are devoid of antibiotic activity.

Earlier reports of 3-methylenecephams include an isolation of methyl 7-phenoxyacetamido-3-methylenecepham-4-carboxylate as a minor product with 7-phenoxyacetamidodeacetoxycephalosporin methyl ester from ring expansion of penicillin V sulfoxide methyl ester.[6a] More recently Ochiai, *et al.*, published a reductive cleavage of the acetoxy group in cephalosporanic acids using chromium(II) salts that led to 3-methylenecephams in quite respectable yield.[6f]

The starting materials (**4**) were prepared by known procedures.[7] A variety of cephalosporanic acids were treated with selected nucleophiles (such as thiourea, thiobenzoic acid, potassium ethyl xanthate, and sodium thiosulfate) in neutral, aqueous solutions at 50° for 20 hr. Two separate reductive cleavages of the $CH_2$–S bond at C-3 in **4** were conducted.

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