

## SESQUITERPENOIDS FROM ACTINOMYCETES: CADIN-4-ENE-1-OL

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**Abstract**—A sesquiterpenoid from *Streptomyces* sp. B-7 is cadin-4-ene-1-ol. It is the enantiomer of epicubenol from cubeb oil.

### INTRODUCTION

TERPENOIDS are generally considered to be higher plant products although examples of every class are known to be present in microorganisms, especially the fungi. The occurrence of terpenoids in lower plants has been especially useful for biosynthetic studies,<sup>1</sup> although many of these terpenoids have structures which, at first, seem complex and unusual: for example, helminthosporal,<sup>2</sup> gibberellic acid<sup>3</sup> and the sesterterpenoids.<sup>4</sup>

Recently, we isolated geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol)<sup>5</sup> and methylisoborneol (1,2,7,7-tetramethyl-2-norbornanol)<sup>6</sup> from several actinomycetes. Generally, the whole broth from shake flask fermentations is distilled and the distillate extracted with CH<sub>2</sub>Cl<sub>2</sub>. The concentrated CH<sub>2</sub>Cl<sub>2</sub> extract is examined by GLC and usually, if geosmin and/or methylisoborneol is present, one or more peaks are observed at higher retention times. Up to now we have isolated five distinct sesquiterpene monoalcohols from different streptomycetes.

### RESULTS AND DISCUSSION

The first sesquiterpenol was obtained from *Streptomyces* sp. B-5a and B-7, both isolated from soil here by M. P. Lechevalier, and from sp. 100-1, received from Jacob Eren and isolated from a reservoir in Israel. All three strains produce geosmin; the Eren strain also yields methylisoborneol. Strain B-7 was chosen for volume production since it also furnished an additional minor product.

The major oily product (I), was usually obtained in yields of 5 mg/l. of whole broth. When pure, it showed O—H absorption but no strong olefinic absorption in the i.r. spectrum. However, the significant end absorption in the u.v. and its ready reaction with I<sub>2</sub> vapor on TLC plates suggested a double bond. The mass spectrum indicated C<sub>15</sub>H<sub>26</sub>O,

<sup>1</sup> P. BERNFELD (editor) *Biogenesis of Natural Compounds*, p. 743, 1025, Pergamon Press, Oxford (1967). W. PARKER, J. S. ROBERTS and R. RAMAGE, *Quart. Rev. London* **21**, 331 (1967).

<sup>2</sup> P. DEMAYO and R. E. WILLIAMS, *J. Am. Chem. Soc.* **87**, 3275 (1965).

<sup>3</sup> P. W. BRIAN, J. F. GROVE and J. MACMILLAN, *Prog. Chem. Nat. Prod.* **18**, 350 (1960).

<sup>4</sup> J. MACMILLAN, *Ann. Rep. Chem. Soc.* **63**, 451 (1966); A. B. TURNER, *Ann. Rep. Chem. Soc.* **65**, 426 (1968).

<sup>5</sup> N. N. GERBER, *Tetrahedron Letters* 2971 (1968).

<sup>6</sup> N. N. GERBER, *J. Antibiotics, Tokyo* **22**, 508 (1969).

since it contained a small molecular ion at 222 (0.5%) and typical sesquiterpene alcohol peaks:<sup>7</sup> M—15 (=CH<sub>3</sub>), M—17 (=OH), M—18 (=H<sub>2</sub>O), M—43 (=C<sub>3</sub>H<sub>7</sub>), M—CH<sub>3</sub> and H<sub>2</sub>O, M—C<sub>3</sub>H<sub>7</sub> and H<sub>2</sub>O.

Selenium dehydrogenation furnished a small amount of cadalene (1,6-dimethyl-4-isopropyl-naphthalene) identical with an authentic sample and a major product with u.v. maxima at 272 and 280 nm indicating a substituted benzene ring. An accurate NMR spectrum, which showed 2 not 3 aromatic protons, established this major product as calamenene (1,6-dimethyl-4-isopropyl-1,2,3,4-tetrahydronaphthalene) and not the isomer in which the other ring is aromatized. The NMR spectrum of (I) showed no CH—OH, one HC=C, 3 methyl doublets at 0.80, 0.87, and 0.95  $\delta$  in CDCl<sub>3</sub> (0.78, 0.85 and 1.08  $\delta$  in C<sub>6</sub>H<sub>6</sub>) and a slightly broadened methyl singlet at 1.68  $\delta$ , CH<sub>3</sub>—C=C. The above facts can be accommodated by four different cadineneols, exclusive of stereochemistry: cadin-3 or 4-ene-1 or 6-ol.

Therefore, the double bond was hydroxylated with osmium tetroxide. The crystalline triol (II), cleaved with NaIO<sub>4</sub>, gave a substance readily dehydrated during GLC to (III). The mass spectrum of (III) verified its formulation as C<sub>15</sub>H<sub>24</sub>O<sub>2</sub>: M = 236 (13%), M—CH<sub>3</sub>, M—C<sub>3</sub>H<sub>7</sub>, and strong peaks involving loss of acetone. Dehydration also occurred during preparation of the 2,4-dinitrophenylhydrazone (DNP) in acid solution. The u.v. spectrum of this derivative showed, by its intensity, that it was a *di* DNP, and by its max at 380 nm that at least one of the chromophores included a conjugated olefinic bond.<sup>8</sup> Thus the hydroxyl group in (I) must be  $\beta$  to the original double bond. If it were  $\alpha$  there would be loss of formaldehyde in the periodate reaction; if it were  $\gamma$  it would not so readily form an  $\alpha,\beta$  unsaturated carbonyl system.

When (II) was oxidized with NaIO<sub>4</sub> and KMnO<sub>4</sub>,<sup>9</sup> a neutral oil was obtained after column chromatography. It had no u.v. absorption, C=O but no O—H in the i.r.; the NMR spectrum showed 3 methyl doublets similar to those in (I), no HC=C, no CH<sub>3</sub>—CO and a sharp methyl singlet at 1.62  $\delta$  (1.45  $\delta$  in C<sub>6</sub>H<sub>6</sub>). The oil gave positive reaction for carbonyl and ester groupings.<sup>10</sup> These properties are best explained by (IV), a hemiacetallactone; this type of 4-keto-1,3-dioxane ring is known.<sup>11</sup> On GLC, (IV) thermally decomposed to a substance of lower retention time. Thus, with the injection port at 240° there was a broad band at 15–19 min; with the injection port at 300° a sharp peak was seen at 15 min followed by tailing. The mass spectrum of material represented by the sharp peak showed M = 208 (20%), C<sub>14</sub>H<sub>24</sub>O, indicating the loss of both CO<sub>2</sub> and H<sub>2</sub>O. Structure (V) is in accord with the base peak at 107 (M—C<sub>3</sub>H<sub>7</sub> and CH<sub>3</sub>COCH<sub>3</sub>) as well as the M—C<sub>3</sub>H<sub>7</sub>, M—C<sub>3</sub>H<sub>7</sub> and H<sub>2</sub>O, M—C<sub>3</sub>H<sub>7</sub> and CH<sub>3</sub> peaks. The only possible alternate to (I), cadin-3-ene-6-ol would furnish a hemiacetallactone (VI) with a primary COOH group which would not be expected to decarboxylate readily.

Two cadin-4-ene-1-ols are known, cubenol and epicubenol, from commercial cubeb oil<sup>12</sup> and from *Cedrela toona* Roxb. timber.<sup>13</sup> The properties of (I) were in complete agreement with those published for epicubenol except for the optical rotation: (I) has  $\alpha_D^{25} = +82^\circ$

<sup>7</sup> Y. HIROSE, *Shitsuryo Bunseki* **15**, 162 (1967); H. C. HILL, R. I. REED and M. T. ROBERT-LOPES, *J. Chem. Soc. (C)*, 93 (1968).

<sup>8</sup> A. I. SCOTT, *Ultraviolet Spectra of Natural Products*, p. 78, MacMillan, New York (1964).

<sup>9</sup> E. VON RUDLOFF, *Can. J. Chem.* **34**, 1413 (1956).

<sup>10</sup> F. FIEGL, *Spot Tests*, Sixth Edition, p. 250, Elsevier, New York (1960).

<sup>11</sup> *Chem. Abs.* indexes it as a  $\delta$  lactone under propionic acid, 3-hydroxymethoxy.

<sup>12</sup> Y. OHTA and Y. HIROSE, *Tetrahedron Letters* 2073 (1967).

<sup>13</sup> B. A. NAGASAMPAGE, L. YANKOV and SUKH DEV, *Tetrahedron Letters* 1913 (1968).

in MeOH, epicubenol has  $\alpha_D^{26} = -96^{12}$  or  $\alpha_D^{30} = -100^{13}$  in  $\text{CHCl}_3$ . Indeed, the stereochemistry of (I) at carbons 1, 6 and 7 was deduced independently, considering the steric requirements of (IV) and the fact that the broad *singlet* NMR band for  $\text{CH}=\text{C}$  in (I) indicates *trans* ring fusion.<sup>13</sup>

A direct comparison of (I) and epicubenol showed identical  $R_T$  in GLC, identical i.r. spectra and identical crystalline triols (II), m.p. 135–138° with opposite rotations,  $\alpha_D^{25} = +23^\circ$  and  $-22^\circ$  both  $\pm 4^\circ$ . Thus (I) and epicubenol are enantiomers; (I) smells “earthy” or “woody”, epicubenol is “sweet-spicy” and seems to have a more intense odor.

## EXPERIMENTAL

NMR spectra were taken in  $\text{CDCl}_3$  unless specified otherwise. Mass spectra were obtained with a Hitachi-Perkin-Elmer RMU-7 using an ionization potential of 70 eV. All GLC was carried out on an F and M model 700 dual column machine equipped with a thermal conductivity detector and a  $183 \times 0.635$  cm column of 10 per cent SE-30 on Diatoport 60–80 mesh using He flow at 50 ml/min. For column chromatography, Mallinkrodt SilicAR CC-7 100–200 mesh was used unless otherwise noted; TLC was carried out on  $2 \times 4$  in. pieces of Eastman chromatogram.

### Preparation and Purification of (I)

*Streptomyces* sp. B-7 was maintained on yeast dextrose (YD) agar slants incubated at 28° until well grown, then refrigerated for storage. For production, 3–4-day-old slants were used to inoculate seed flasks (50 ml YD/250 ml Erlenmeyer flask). After 2–3 days at 28° with rotary shaking at 220 rev/min, whole broth from these flasks was inoculated at 5% into SBM/J (10 g soybean meal, 10 g Wilson's peptone No. 851, 20 g Cerelease, 5 g  $\text{NaCl/l}$ , 250 ml/2 l flask, pH 7.5 before autoclaving). After 5 days at 28° with reciprocal shaking at 80 strokes/min, the whole broth was distilled at atmospheric pressure. A typical batch had 40 flasks, 10 l. of whole broth yielding 4 l. of clear distillate which was extracted 2 times with a 1/10 vol. of  $\text{CH}_2\text{Cl}_2$ . The concentrated extract was assayed by GLC then the combined extracts from four batches applied to a 40-g silica column, 200–325 mesh, eluting with  $\text{CH}_2\text{Cl}_2$ . Fractions were assayed by GLC and pure (I) obtained by preparative GLC. The retention times at 180° were: geosmin 4.8, minor product 8.6, (I) 10.3 min. Later it was found that column fractions could be monitored by TLC in benzene– $\text{CHCl}_3$  (4:1). Using  $\text{I}_2$  vapor, (I) was detected at  $R_f$  0.30; geosmin and the minor product were not visualized. With a second 40-g silica column, the (I)-containing fractions furnished 95 per cent pure (I) without preparative GLC.

### Selenium Dehydrogenation of (I)

Under our conditions, commercial guaiene gave only S-guiazulene (1,4-dimethyl-7-isopropylazulene). There was no evidence for any thermal isomerization to Se-guiazulene (2,4-dimethyl-7-isopropylazulene) which others have observed with somewhat different conditions.<sup>14</sup> Pure (I), 30 mg and 50 mg of Se-powder in the bottom of a  $14.6 \times 0.635$  cm (ID) pyrex tube was heated at 310° (bath temperature) for 3 hr under  $\text{CO}_2$ . The tube was inserted through a hole in the heavy asbestos cover of the sand bath, leaving the upper half of the tube cool. After cooling, the cyclohexane soluble portion was chromatographed on 10 g of silica, eluting with cyclohexane. Fractions were assayed by TLC where cadalene was visible as a blue fluorescent spot in u.v. light,  $R_f$  0.5. The cadalene obtained by preparative GLC had a retention time (12 min at 160°, programmed 4°/min) and u.v. spectrum (max at 291, 326 nm) identical with an authentic sample. The main product was dark in u.v. light, had an  $R_f > 0.5$  on TLC, retention time 8.8 min and u.v.<sub>max</sub> 272, 280 nm. Its NMR spectrum showed three methyl doublets at 0.7, 0.98 and 1.22  $\delta$ , an aromatic methyl at 2.23  $\delta$  and a band whose area indicates four tetralin type protons at 1.7  $\delta$ .

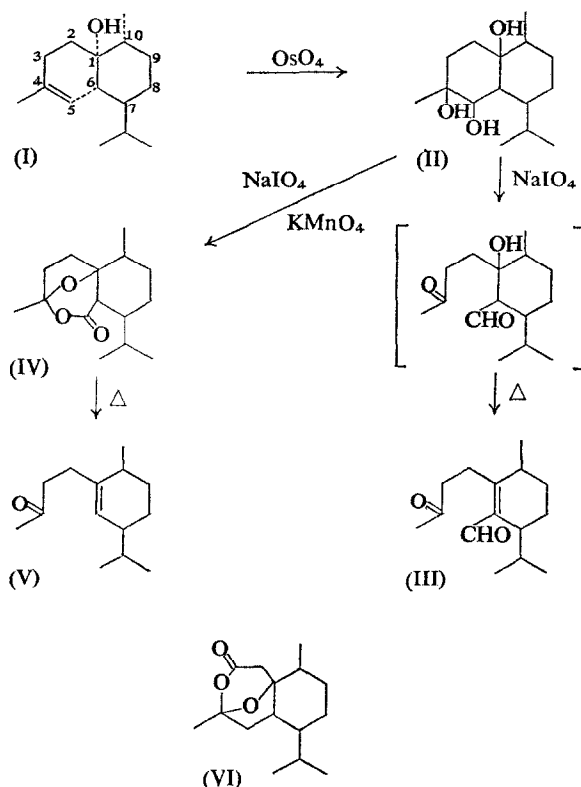
### Preparation of Triol (II)

Pure (I), 50 mg, in 5 ml of cyclohexane and 0.5 ml pyridine was treated with 9 ml of an  $\text{OsO}_4$  solution (1 g in 100 ml of cyclohexane). After 2 days in the dark at room temp., the tan, osmate ester complex was filtered, washed with cyclohexane then saponified by shaking overnight at 28° with 1 g of KOH and 2 g of mannitol in 25 ml of water. The crystalline triol, m.p. 137–8° was obtained by  $\text{CHCl}_3$  extraction. Its NMR spectrum showed an integral area equivalent to 9 H in the 0.6–1.0  $\delta$  region, and a clear  $\text{CH}_3\text{—C—OH}$  at 1.3  $\delta$ .

<sup>14</sup> H. A. SILVERWORTH and M. ORCHIN, *J. Org. Chem.* **27**, 3401 (1962); Y. OTANE, H. OKAMOTO and I. OGURA, *Yakugaku Zasshi* **88**, 807 (1968), *Chem. Abstr.* **70**, 47614 p (1970).

*Periodate Cleavage of (II)*

Triol from 25 mg of (I) was dissolved in 10 ml of 30% aq. pyridine and 100 mg solid  $\text{NaIO}_4$  added. The mixture was shaken and  $\text{CO}_2$  periodically bubbled through. During 24 hr, no volatile carbonyl compounds could be trapped in a 10% HCl solution of 2,4-dinitrophenylhydrazine. Control runs with 10 mg of glycerol



or propylene glycol readily gave formaldehyde and acetaldehyde DNP derivatives. The reaction mixture was acidified and extracted with  $\text{CHCl}_3$ . This furnished a crystalline residue, m.p.  $95\text{--}105^\circ$ , whose i.r. spectrum showed strong  $\text{O—H}$  and  $\text{C=O}$  bands. The NMR spectrum had an integral equivalent to 9 H in

the  $0.7\text{--}1.0\ \delta$  region and a clear  $\text{CH}_3\text{—C}(=\text{O})$  at  $2.3\ \delta$ . It gave a single peak in analytical GLC and the red DNP derivative melted at  $233\text{--}5^\circ$ .

*Periodate-Permanganate Oxidation of (II)*

Triol from the hydrolysis of 100 mg osmate ester complex was treated with pyridine (4 ml) and 10 ml of a solution containing 100 mg  $\text{KIO}_4$  and 20 mg  $\text{KMnO}_4$ . After several days shaking at  $28^\circ$ , the mixture was acidified and clarified by the gradual addition of small amounts of solid sodium metabisulfite. The crude product obtained by  $\text{CHCl}_3$  extraction was purified on a 30 g silica CC-4 column. Elution with  $\text{EtOAc-CHCl}_3$  (1:9) furnished 18 mg of a neutral, mobile oil (IV). When the eluate was changed to  $\text{EtOAc-CHCl}_3$  (1:1) acidic products (13 mg) were obtained. To test for a carbonyl group  $2\text{--}3\ \mu\text{g}$  of (IV) was spotted on a TLC plate from  $\text{CHCl}_3$  solution (a  $\text{CHCl}_3$  control was negative but some other solvents gave positive tests) and immediately overspotted with  $10\text{--}20\ \mu\text{g}$  of DNP from a solution in 10% HCl. After 2–3 min at  $50^\circ$  in an oven, the plate was run in  $\text{CHCl}_3$ . The unchanged reagent remained near the origin while many DNP derivatives including those of (IV) and acetophenone had  $R_f > 0.7$ .

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