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Epicubebol and Related Sesquiterpenoids from the Brown Alga *Dictyopteris divaricata*[1]

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Epicubebol has been isolated from the methanol extracts of the brown alga *Dictyopteris divaricata* as the major constituent. Cadinane-type sesquiterpenes, cubebenes, δ-cadinene, cubenol, and epicubenol, as well as two sesquiterpene methyl ethers as minor constituents have also been obtained from the extracts.

Recently many interesting secondary metabolites such as diterpenes[2,3] and sesquiterpene-substituted hydroquinones[4] have been obtained from the brown algae of the family Dictyotaceae. This prompted us to reinvestigate the constituents of the brown alga *Dictyopteris divaricata* (Okam.) Okamura ("Ezoyahazu" in Japanese) in this family.[5] We wish to report the isolation and structures of several cadinane-type sesquiterpenoids from this alga.

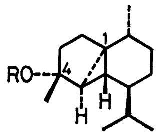
The neutral methanol extracts, obtained from freshly collected algae, were fractionated by column chromatography over neutral alumina. The fraction eluted with hexane gave a mixture of sesquiterpene hydrocarbons consisting of (−)-α-cubebene (**7**),[6] (−)-β-cubebene (**8**),[6] (+)-δ-cadinene (**9**), and other hydrocarbons. The fraction eluted with benzene was subjected to a combination of column and thin-layer chromatography over silica gel to yield compounds **3**, **4**, **5**, and **6** as minor components. The fraction eluted with ether was subjected to repeated thin-layer chromatography to give compound **1** as the major component.

Compound **1**, colorless oil, $[\alpha]_D^{21}$ −42°, was analyzed for $C_{15}H_{26}O$ by mass spectroscopy *m/e* 222 (M^+). Its IR spectrum showed hydroxyl absorption at ν_{max} 3380 cm^{-1}, and its ^1H NMR spectrum signals due to two cyclopropane protons at δ 0.35 and 0.77 (each 1H, m), a tertiary methyl group at 1.25 (3H, s), and three secondary methyl groups at 0.90, 0.93, and 0.99 (each 3H, d, $J=7$ Hz), two of which being ascribable to the isopropyl group on the basis of the base peak at *m/e* 161 $[M^+-H_2O-CH(CH_3)_2]$ in the mass spectrum of **1**. The tertiary nature of the hydroxyl group was shown by its resistance to acetylation with acetic anhydride in pyridine and by the presence of a quaternary carbon at δ 80.0 ppm in the ^{13}C NMR spectrum. Since the ^{13}C NMR spectrum indicates no sp^2 carbon, compound **1**, having three degrees of unsaturation, should be a tricyclic sesquiterpene alcohol including a cyclopropane ring.

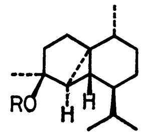
Treatment of **1** with $SOCl_2$ in CH_2Cl_2–pyridine at -7—-9 °C gave (−)-α-cubebene (**7**), (−)-β-cubebene (**8**), and (+)-δ-cadinene (**9**) in a low yield. On the other hand, treatment with TsOH in benzene at room temperature afforded (+)-δ-cadinene (**9**) as a major product and (−)-zonarene (**10**)[7] as a minor one. This indicates that compound **1** is cubebol (**2**), previously isolated from the oil of cubeb *Piper cubeba* L.,[8,9] or epicubebol (**1**) synthesized from (−)-α-cubebene (**7**) by oxymercuration-demercuration reaction.[9] Comparison of the spectral data show **1** to be identical with epicubebol (**1**). Isolation of epicubebol (**1**) as a natural product seems to have been carried out for the first time.

Compounds **3** and **4** were found to be identical with epicubenol (**3**) and cubenol (**4**), respectively, which have also been isolated from the cubeb oil,[10] by comparison of spectral data. **3** and **4** were derived from **1** on treatment with TsOH in benzene and also with dil HCl in dioxane–water[11] (see Experimental).

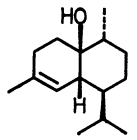
Compound **6**, $C_{16}H_{28}O$ (*m/e* 236; M^+), $[\alpha]_D^{24}$ −23°, displayed a ^1H NMR spectrum very similar to that of epicubebol (**1**) except for the signal due to the methoxyl group at δ 3.13 (3H, s), suggesting that **6** would be the methyl ether derivative of **1**. Spectral similarity between compounds **5** and **6** indicates that **5** would be the stereoisomer at C-4 of **6**, *viz.* the methyl ether derivative of cubebol (**2**). This was confirmed by the following reaction. Treatment of **1** with methanol containing a catalytic amount of H_2SO_4 at room temperature resulted in the formation of **5** (13%) and **6** (13%), suggesting that these methyl ethers are artifacts which were generated during the course of extraction. Thus, extraction of the alga was performed with ether instead



1: R=H
6: R=CH_3



2: R=H
5: R=CH_3



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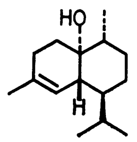

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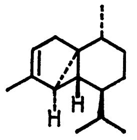

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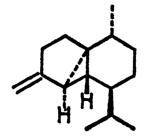

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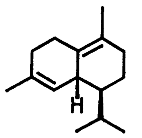

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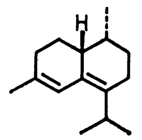

10

of methanol, no methyl ether derivatives, **5** and **6**, being obtained from the ether extracts as expected.

In previous studies,[5] no epicubebol (**1**), which exists as the major component of the extracts in the present study, was isolated. In contrast, no dictyopterols, dictyopterone, δ-cadinol, and other sesquiterpenes except for (−)-α-cubebene[12] could be detected in the present study. The difference in the results between the present and the previous studies seems to depend mainly upon the isolation procedure. In the previous studies, after distillation of the extracts with steam, fractional distillation was employed for fractionation of the neutral components, whereas we have treated the extracts at low temperature (below 35 °C) and further with rapid column and thin-layer chromatography. On heating at *ca.* 130 °C, epicubebol (**1**) gave (−)-α-cubebene, (−)-β-cubebene, and (+)-δ-cadinene together with (−)-*trans*-calamenene. By prolonged contact with silica gel, **1** turned into (+)-δ-cadinene, (−)-zonarene, and other hydrocarbons.

Recently 4,10-epoxymuurolane, which appears to be derived from epicubebol, has been isolated from the brown alga *Dilophus fasciola* together with several cadinane-type sesquiterpenes.[13]

It is interesting that the marine alga produces epicubebol (**1**) instead of cubebol (**2**) which has been isolated from the terrestrial plant.

Experimental

IR spectra were measured on a JASCO A-102 spectrometer and ^{1}H NMR spectra on a JEOL JNM-PS-100 spectrophotometer, TMS being used as an internal reference in CCl_4. The ^{13}C NMR spectrum was obtained with a Bruker SXP4-100 spectrophotometer in $CDCl_3$. The optical rotations were measured in $CHCl_3$. Aluminum oxide (Merck, activity II—III) and silica gel (Merck, Kieselgel 60, 70—230 mesh) were used for column chromatography. Silica gel (Merck, Kieselgel GF_{254} (Type 60)) was used for preparative thin-layer chromatography (PTLC).

Isolation. *Dictyopteris divaricata* was collected at Oshoro Bay, Hokkaido, during the months April—August 1980. No distinct difference was observed between the extracts in each collection. Half-dried alga (2.2 kg), collected in April, was extracted with methanol, and the resulting solution was concentrated under reduced pressure. The residue was percolated with ether, and the ether solution was shaken with 5% $NaHCO_3$ and saturated brine, and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, a neutral oil (104 g) was obtained. This was fractionated by column chromatography over neutral alumina. Elution with hexane gave a mixture of sesquiterpene hydrocarbons from which (−)-α-cubebene (**7**) (0.04% of the neutral oil), (−)-β-cubebene (**8**) (1%), and (+)-δ-cadinene (**9**) (0.8%) were detected by comparison of the retention times in GLC with those of authentic samples. Elution with benzene gave an oily substance which was subjected to a combination of silica-gel column and PTLC to yield **3** (0.1%), **4** (0.02%), **5** (1.5%), and **6** (1.5%) (in order of decreasing porality) along with an unidentified alcohol. Elution with ether gave a mixture of alcohols which was repeatedly chromatographed on PTLC to yield **1** (13%) together with phytols, cholesterol, and at least two unidentified alcohols.

1: Colorless oil; $[\alpha]_D^{21}$ −42° (*c* 1.54); IR, ν_{max} (film) 3380, 1370, 1180, 1110, and 910 cm^{-1}; ^{1}H NMR, δ 0.35, 0.77 (each 1H, m), 0.90, 0.93, 0.99 (each 3H, d, J=7 Hz) and 1.25 (3H, s); ^{13}C NMR, δ 80.8 (s), 44.6 (d), 39.9 (d), 36.6 (t), 34.9 (s), 33.6 (d), 31.8 (d), 30.2 (t), 29.8 (t), 27.0 (t), 25.3 (d), 25.0 (q), 20.0 (q), 19.8 (q), and 19.1 (q); MS, *m/e* (relative intensity) 222 (12), 207 (83), 204 (9), and 161 (100). The IR (film and CCl_4) and ^{1}H NMR ($CDCl_3$) spectra were consistent with those of epicubebol.

3: Colorless oil; $[\alpha]_D^{25}$ −96° (*c* 0.94); The spectral properties were identical with those reported [10] for epicubenol.

4: Colorless oil; $[\alpha]_D^{20}$ −33° (*c* 0.13); The spectral properties were identical with those reported[10] for cubenol.

5: Colorless oil; $[\alpha]_D^{23}$ −82° (*c* 1.36); IR, ν_{max} (film) 1370, 1310, 1205, 1140, 1070, and 885 cm^{-1}; ^{1}H NMR, δ 0.74 (1H, m), 0.91 (6H, d, J=6 Hz), 0.96 (3H, d, J=6 Hz), 1.14 (3H, s), and 3.14 (3H, s); MS, *m/e* 236 (1), 221 (69), 204 (18), 161 (65), and 41 (100).

6: Colorless oil; $[\alpha]_D^{24}$ −23° (*c* 0.60); IR, ν_{max} (film) 1370, 1110, 1090, 1075, and 865 cm^{-1}; ^{1}H NMR, δ 0.35 (1H, m), 0.90, 0.94, 0.96 (each 3H, d, J=7 Hz), 1.21 (3H, s), and 3.13 (3H, s); MS, *m/e* 236 (1), 221 (100), 204 (8), and 161 (57).

*Dehydration of **1** with Thionyl Chloride.* A solution of **1** (107 mg) and thionyl chloride (0.04 ml) in dry dichloromethane (6 ml) and pyridine (1 ml) was allowed to stand at −7—−9 °C for 5 min. The mixture was then poured into ice-cooled water and extracted with ether. The ether solution was washed with saturated brine and dried over anhydrous Na_2SO_4. A residual substance obtained after removal of the solvent was chromatographed on alumina column. Elution with hexane gave a mixture of hydrocarbon (20 mg), GLC of which revealed that the mixture consists of (−)-α-cubebene (**7**) (20%), (−)-β-cubebene (**8**) (1.5%), (+)-δ-cadinene (**9**) (40%), and other hydrocarbons. Elution with ether gave unreacted **1** (25 mg).

*Dehydration of **1** with* p-*Toluenesulfonic Acid.* A solution of **1** (97 mg) and *p*-toluenesulfonic acid (2 mg) in benzene (5 ml) was stirred at room temperature for 30 min. After evaporation of benzene, the residual oily products were subjected to column chromatography on alumina. Elution with hexane gave a mixture of hydrocarbons (87 mg) consisting of (+)-δ-cadinene (**9**) (70%) and (−)-zonarene (**10**) (1%). Purification of (+)-δ-cadinene was carried out by preparative GLC. **10** was identified as (−)-zonarene by comparison of the retention time in GLC with that of an authentic sample. Elution with ether gave a mixture of alcohols which was further chromatographed on PTLC to afford epicubenol (**3**) (4 mg), cubenol (**4**) (2 mg), and **1** (1 mg).

*Conversion of **1** into **3** and **4**.* A small amount of dil HCl was added to a solution of **1** (97 mg) in dioxane (6 ml) and water (2.5 ml), and the mixture was allowed to stand at room temperature for 30 h. Water was added and extraction was carried out with ether. The ether solution was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated to give an oily residue. The residue was chromatographed on alumina column. Fraction eluted with ether was further subjected to repeated PTLC to give epicubenol (**3**) (11 mg), cubenol (**4**) (4 mg), and **1** (24 mg) along with unidentified alcoholic compounds and ketonic compounds.

*Methanolysis of **1**.* A solution of **1** (65 mg) and a catalytic amount of concd H_2SO_4 in methanol (20 ml) was allowed to stand at room temperature for 2 h with continuous stirring. The mixture was then concentrated *in vacuo*. Water was added and extraction was carried out with ether. The ether solution was washed with 5% $NaHCO_3$ and saturated brine, and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residual oil was chromatographed on silica-gel column to give **5** (9 mg), **6** (9 mg), and **1** (44 mg).

*Thermal Reaction of **1**.* **1** (100 mg) was placed in a flask fitted with condenser which was immersed in an oil bath maintained at 130 °C under nitrogen atmosphere. After 5 h the reaction products were separated by column chromatography over alumina to yield a hydrocarbon fraction (54 mg) which was further subjected to preparative GLC to give (−)-α-cubebene (**7**) (21 mg), (−)-β-cubebene (**8**) (11 mg), (+)-δ-cadinene (**9**) (11 mg), and (−)-*trans*-calamenene (4 mg). The structure of *trans*-calamenene was identified by comparison of the IR and ^{1}H NMR spectra with reported[14] for calamenene. Unchanged **1** (33 mg) was recovered.

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