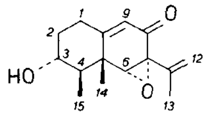

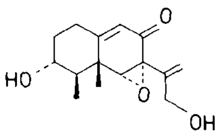

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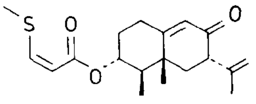

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REFERENCES

1. Peresse, M. and Le Picard, D. (1980) *Mycopathologia* **71**, 23.
2. Trique, B. and Tirilly, Y. (1981) *Agronomie* **1**, 428.
3. Riche, C., Pascard-Billy, C., Devys, M., Gaudemer, A., Barbier, M. and Bousquet, J. F. (1974) *Tetrahedron Letters* **32**, 2765.
4. Bottalico, A., Frisullo, S., Lerario, P., Randazzo, G. and Capasso, R. (1982) *Phytopathol. Mediterr.* **21**, 39.
5. Neuenschwander, M., Neuenschwander, A. and Steinegger, E. (1979) *Chimia* **33**, 50.
6. Neuenschwander, M., Neuenschwander, A., Steinegger, E. and Engel, P. (1979) *Helv. Chim. Acta* **62**, 609.
7. Kranz, J. (1981) in *Microbial Ecology of the Phylloplane* (Blakeman, J. P., ed.) p. 347. Academic Press, London.
8. Keen, N. T., Sims, J. J., Erwin, D. C., Rice, E. and Partridge, J. E. (1971) *Phytopathology* **61**, 1084.

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(+)-8-HYDROXYCALAMENENE: A FISH-POISON PRINCIPLE OF *DYSOXYLUM ACUTANGULUM* AND *D. ALLIACEUM*[*]

MUGIO NISHIZAWA, AKIO INOUE, SETIJATI SASTRAPRADJA† and YUJI HAYASHI

Department of Chemistry, Faculty of Science, Osaka City University, Osaka 558, Japan; †The National Biological Institute, Bogor, Indonesia

(*Revised received* 31 *January* 1983)

Key Word Index—*Dysoxylum acutangulum*; *D. alliaceum*; Meliaceae; fish-poison; antibacterial activity.

Abstract—A fish-poison principle of *Dysoxylum acutangulum* and *D. alliaceum* has been identified as (+)-8-hydroxycalamenene, a new natural sesquiterpene phenol. This compound shows not only significant toxicity against fish but also antibacterial activity.

INTRODUCTION

Seeds of *Dysoxylum acutangulum* have been traditionally known as fish-poisonous plant material in Sumatera, Indonesia. We have investigated the active principles of this plant by monitoring the toxicity against a species of fish, *Oryzias latipes*, and isolated a phenolic sesquiterpene, **1**, as a major toxic constituent. This compound shows a significant fish-toxicity against *Oryzias latipes* at 5 ppm concentration and moderate antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus*, *Candida albicans* and *Trichophyton mentagrophytes*, at 5–20 ppm (MIC). However, it is ineffective against Gram-negative bacteria, such as *Esherichia coli* or *Pseudomonas aeruginosa*.

This article describes the isolation and structure determination of the active principle which is identified as (+)-8-hydroxycalamenene (**1**), a new natural sesquiterpene.

RESULTS AND DISCUSSION

A crude ethanol extract of seeds of *D. acutangulum* showed a significant fish-toxicity, and the activity was always monitored by the bioassay with *Oryzias latipes*. Silica gel CC followed by vacuum distillation afforded a phenolic sesquiterpene (**1**) as a major active constituent. Compound **1**, $C_{15}H_{22}O$, was obtained as a liquid with bp 150–155°/0.1 mmHg and had a phenolic ring (IR ν_{max} cm^{-1}: 3500, 1620, 1580). The ^{13}C NMR spec-

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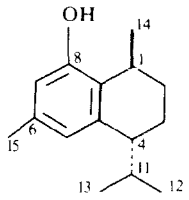
[*] Dedicated to Emeritus Professor Takeo Sakan of Osaka City University on the occasion of his 70th birthday.



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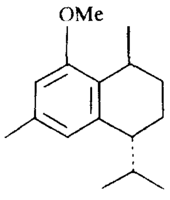

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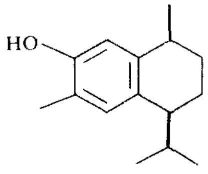

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trum (Table 1) showed the presence of four methyls (δ19.7, 21.1, 21.3 and 22.2), two methylenes (19.2 and 27.2), three methynes (26.7, 33.2 and 43.2) and six aromatic carbons in which two were unsubstituted (113.8 and 123.1) and one at 153.2 carried the phenolic hydroxyl group. This evidence suggested a cadinane-type or closely related carbon skeleton for this compound. In the ^{1}H NMR spectrum, signals due to two aromatic protons appeared at δ6.39 and 6.57 as broad singlets. These chemical shift values are clearly different from those of (−)-7-hydroxycalamenene (**3**) (δ6.52 and 6.92) [1] or its *trans*-isomer (6.62 and 6.92) [2].

Upon irradiation at the aromatic methyl proton (δ2.21), the aromatic proton signals turned to an AB doublet with the coupling constant of 2 Hz (*meta*-coupling). Therefore, the two aromatic protons must be located at the C-5 and C-7 positions, vicinally to the methyl group. Three kinds of secondary methyl signals were observed at δ0.83, 0.97 and 1.20 corresponding to a secondary methyl group and an isopropyl group, which were located at C-1 and C-4 (or reverse) by careful decoupling experiments. Additionally, by consideration of sesquiterpene biogenesis, structure **1** was established for this active principle.

(+)-8-Methoxycalamenene (**2**) has been isolated from a horny coral, *Subergorgia hicksoni* [3], and its absolute configuration was shown to be 1*S*,4*R* by chemical derivation [4]. When (+)-**1** was treated with dimethyl sulfate in basic media, (+)-8-methoxycalamenene (**2**) was produced in good yield. Although the magnitude of the optical rotation of this derivative ($[\alpha]_D^{24}$ +46.1°) was larger than the reported value ($[\alpha]_D^{25}$ +30°), the sign of the rotation, IR and NMR spectral data were completely identical with those of the natural product. Thus, the structure of the active principle was established as (+)-(1*S*,4*R*)-8-hydroxycalamenene. It should be noted that such a simple and easily available natural product (2.60 % yield from seeds) showed a significant biological activity against fish and particular kinds of bacteria. A related species, *Dysoxylum aliaceum*, was also found to contain (+)-8-hydroxycalamenene in a comparable content as an active component of the seeds.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (25 MHz, CDCl$_3$, δ value)

Carbon No.	**1**	**2**
1	26.7 *d*	26.7 *d*
2	27.2 *t*	27.3 *t*
3	19.2 *t*	*
4	43.2 *d*	43.2 *d*
5	123.1 *d*	122.7 *d*
6	135.1 *s*	134.6 *s*
7	113.8 *d*	108.6 *d*
8	153.2 *s*	157.2 *s*
9	126.5 *s*	128.6 *s*
10	141.3 *s*	140.7 *s*
11	33.2 *d*	33.4 *d*
12	19.7 *q*†	19.3 *q*†
13	21.1 *q*†	19.8 *q*†
14	22.2 *q*‡	22.3 *q*‡
15	21.3 *q*‡	21.6 *q*‡
OMe	—	55.2 *q*

*Signal overlapped with one of the methyl signals.

†, ‡Assignments may be interchangable.

EXPERIMENTAL

Isolation of (+)-8-hydroxycalamenene (**1**). Dried seeds (1.15 kg) of *Dysoxylum acutangulum* Miq., collected in Bogor, Indonesia, in April 1981, were crushed with EtOH using a mechanical mixer and the resulting slurry was allowed to stand for 2 months. The extract obtained by filtration through a Celite pad was concd to *ca* 300 ml and to this was added 500 ml H_2O. The mixture was extracted × 3 with hexane (300 ml). The dried extract was filtered and concd to give a brown syrup (59.2 g). This syrup (20 g) was subjected to CC on Si gel (200 g) eluted with petrol–EtOAc (15:1). The main constituent was eluted in early fractions to give 12.5 g of yellow syrup, which was essentially homogeneous on TLC. Vacuum distillation (150–160°/0.1 mm Hg) using a bulb-to-bulb distillation apparatus gave pure (+)-8-hydroxycalamenene (**1**) (9.85 g, 2.6 % yield) as a pale yellow liquid: $[\alpha]_D^{15}$ +38.0° (CHCl$_3$; *c* 1.37); IR ν_{max}^{Neat} cm^{-1}: 3500, 1620, 1580, 1460, 1420, 1384, 1368, 1340, 1320, 1290, 1240, 1205, 1160, 1120, 1100, 1060, 1030, 975, 900, 840, 760; ^{1}H NMR (CDCl$_3$): δ0.83 (3H, *d*, $J = 7$ Hz), 0.97 (3H, *d*, $J = 7$ Hz), 1.20 (3H, *d*, $J = 7$ Hz), 1.52 (1H, *m*), 1.75–2.10 (4H, *m*), 2.21 (3H, *s*), 2.46 (1H, *m*), 3.07 (1H, *m*), 5.05 (OH), 6.39 (1H, *br s*), 6.57 (1H, *br s*); MS *m/z* (rel. int.): 218 $[M]^+$ (17), 175 [M − *iso*-Pr]$^+$ (100), 160 $[175 - Me]^+$ (7). (Calcd for $C_{15}H_{22}O$: C, 82.51; H, 10.16. Found: C, 82.46; H, 10.16 %.)

The same active component, **1**, was also obtained from the seeds of *D. alliaceum* in 3.8 % yield by the same procedure.

(+)-8-*Methoxycalamenene* (**2**). To a soln of (+)-8-hydroxycalamenene (**1**) (191 mg, 0.88 mmol) in 15 % aq. NaOH (20 ml) was slowly added Me_2SO_4 (0.51 g, 4.00 mmol), and the resulting mixture was stirred at room temp. overnight. To this was added H_2O (40 ml), and following extraction with CH_2Cl_2 gave crude product (189 mg). CC on Si gel (30 g) with hexane afforded the pure methyl ether, **2** (136 mg, 67 % yield); $[\alpha]_D^{26}$ +46.1° (CHCl$_3$; *c* 1.11); IR, ^{1}H NMR and ^{13}C NMR data were fully identical with the reported values [3]. (Calcd for $C_{16}H_{24}O$: C, 82.70; H, 10.41. Found: C, 82.60; H, 10.48%.)

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REFERENCES

1. Rowe, J. W. and Toda, J. K. (**1969**) *Chem. Ind.* 922.
2. De Mayo, P., Williams, R. E., Buchi, G. and Feairheller, S. H. (1965) *Tetrahedron* **21**, 619.
3. Kashman, Y. (1979) *Tetrahedron* **35**, 263.
4. Bunko, J. D., Ghisalberti, E. L. and Jefferies, P. R. (1981) *Aust. J. Chem.* **34**, 2237.

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6-HYDROXYTOXOL ESTERS AND A *SECO*-DAMMARANE DERIVATIVE FROM *ABROTANELLA FORSTERIOIDES**

HARALD GREGER, CHRISTA ZDERO† and FERDINAND BOHLMANN†

Institute for Botany, University of Vienna, A-1030 Vienna, Austria; †Institute for Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, West Germany.

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Key Word Index—*Abrotanella forsterioides*; Compositae; *p*-hydroxyacetophenone derivatives; toxol derivatives; triterpenes; *seco*-dammarane derivative.

Abstract—*Abrotanella forsterioides* afforded euparin, 6-hydroxytremetone and three 6-hydroxytoxol esters, one of them not being isolated previously. Furthermore a *seco*-triterpene was isolated. The tribal position of the genus *Abrotanella* in the Compositae is still an unsolved problem. Morphological investigations suggest that this genus should be transferred from the tribe Anthemideae to the Senecioneae [1, 2]. So far two species have been studied chemically; one afforded *ent*-kaurane derivatives, while both contained euparin and hydroxytremetone [3].

A reinvestigation of the roots of *Abrotanella forsterioides* Hook. fil. afforded, in addition to euparine (**1**) and 6-hydroxytremetone (**2**), the 6-hydroxy-3-epi-toxol esters **3**–**5** [4]. The senecioate **5** has not been isolated previously; its structure could be deduced from the ^{1}H NMR spectrum, which was close to that of **4** (see Experimental). Furthermore several triterpenes were present. In addition to stigmasterol, sitosterol and Δ^{12}-lupeol, a new *seco*-triterpene acetate was isolated. The molecular formula was $C_{32}H_{54}O_2$, while the ^{1}H NMR spectrum (Table 1) showed that four olefinic methyls were present, which required at least two double bonds. Thus, only three rings were present indicating a *seco*-triterpene. Accordingly, the signal of an acetoxymethylene group was apparent (4.03 *t*) and only one olefinic proton (5.09 *br t*). Three additional methyl singlets and one doublet indicated that a dammarane derivative may be present. Careful spin decoupling also, after addition of Eu(fod)$_3$, showed that a 4,5-double bond was very likely. Irradiation of the olefinic methyls caused a sharpening of one of the allylic threefold doublets (H-6), while both were coupled with a multiplet at δ 1.55 (H-7). After addition of Eu(fod)$_3$, the signals of the protons β to the acetoxy group could be assigned. Their multiplicity excluded any other position for the acetate group. The Eu(fod)$_3$-induced shifts further showed that the allylic proton were near the acetate group. Small differences in the shifts of the methyl singlets agreed with the assumption that a *seco*-dammarane was more likely than a euphane derivative. All data, therefore, agreed best with structure **6**. This proposal was further supported by the fragmentation pattern in the mass spectrum of **6**. A prominent peak m/z 287 could be explained as an elimination of both side chains ($-C_6H_{11}$ and $-C_5H_8O_2$, McLafferty). Probably **6** was formed via **7**. The aerial parts also afforded **1**–**5**.

The chemistry of *A. forsterioides* clearly showed that this genus cannot be placed in the tribe Anthemideae. Its position in the Senecioneae is supported by the occurrence of hydroxytoxol esters **3**–**5**. This type of *p*-hydroxyacetophenone derivative has been reported from nine *Senecio* species [4], though these compounds are also present in a few genera in other tribes.

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*Part 460 in the series "Naturally Occurring Terpene Derivatives". For Part 459 see Bohlmann, F., Zdero, C., Jakupovic, J., King, R. M. and Robinson, H. (1983) *Phytochemistry* (in press).