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TWO NEW SESQUITERPENE ESTERS FROM *ARMILLARIA MELLEA*

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Key Word Index—*Armillaria mellea*; basidiomycete; secondary metabolites; sesquiterpene aryl esters.

Abstract—Two new sesquiterpene aryl esters 15-hydroxy-5′-*O*-methylmelledonal and 5′-*O*-methylmelledonal, were isolated from the culture broth extract of *Armillaria mellea*. Their structures were deduced from chemical and spectral data.

INTRODUCTION

The biologically active metabolites isolated from the pathogenic basidiomycete *Armillaria mellea* comprise two major structural types represented by armillyl orsellinate (**1**) [1] and melleolide (**2**) [2]. The protoilludane skeleton present in both **1** and **2** is indicative of a common biosynthetic pathway. Recently we reported the isolation of melledonal (**3**) and melledonol (**4**), a group of compounds possessing the melleolide-type structural framework [3]. We now report the isolation of two new sesquiterpene aryl esters for which the structures 15-hydroxy-5′-*O*-methylmelledonal (**5**) and 5′-*O*-methylmelledonal (**6**) are proposed. Compound **5** is the first metabolite from *A. mellea* to possess oxygenation of the *gem*-dimethyl groups.

RESULTS AND DISCUSSION

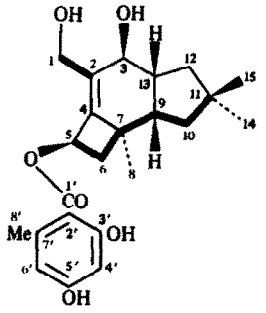
The molecular formula $C_{24}H_{30}O_9$ of 15-hydroxy-5′-*O*-methylmelledonal (**5**), mp 174–176°; $[\alpha]_D^{25}+77.3°$ (MeOH; *c* 0.22) was established by elemental analysis and CIMS ($[MH^+]$, *m/z* 463; base peak at *m/z* 165 corresponds to the everninate group [4]). The IR spectrum (KBr) of **5** registered two carbonyl bands at 1688 (unsaturated aldehyde) and 1641 cm^{-1} (chelated ester) and hydroxyl bands at 3440 cm^{-1}. The 270 MHz ^1H NMR spectrum ($CDCl_3$) showed signals due to an α,β-unsaturated aldehyde group (δ9.53, *s*, CHO; 6.96, *s*, vinylic) and a methoxyl group (3.78, *s*, OMe). Compound **5** possessed only two (δ0.99, 1.43) of the three aliphatic methyl groups characteristic of melledonal (**3**). The appearance of an AB quartet at δ3.61 (*gem* coupling *J* = 11.63 Hz, 2H) indicates that one of the two geminal methyl groups has undergone oxygenation. Decoupling experiments enabled the assignment of the remaining protons as follows: δ2.08, 2.12 (2H, 2 × *d*, *J* = 14 Hz, H-12α,β), 2.01 (1H, *dd*, *J* = 10.8, 8.2; H-6α), 2.17 (1H, *dd*, *J* = 10.8, 9.0; H-6β), 2.31 (3H, *s*, Me-8′), 2.59 (1H, *d*, *J* = 3.7, H-9), 3.9 (1H, *d*, *J* = 3.7, H-10), 5.73 (1H, *t*, *J* = 8.8, H-5), 6.22 (1H, *d*, *J* = 2.5, H-4′), 6.31 (1H, *d*, *J* = 2.5, H-6′), 11.64 (1H, *s*, phenolic OH).

The ^{13}C NMR spectrum (67.8 MHz, $CDCl_3$) was consistent with the proposed structure, the relevant signals being at δ196.1 (C-1), 153.71 (C-3), 135.2 (C-2), 79.6 (C-10), 77.0 (C-4), 74.8 (C-13), 73.7 (C-5), 55.4 (C-9), 50.7 (C-12), 35.7 (C-7), 31.8 (C-6), 20.9 (C-14), 18.6 (C-8). The nine resonances of the everninate group compared well with those of methyl everninate [4]. A peak at δ69.9 was indicative of the primary hydroxyl function at C-15. Comparison of NMR data of compound **5** with that of **3** indicated a similar structure for both.

A dibenzoate derivative (**7**) of **5** was formed (BzCl/pyridine; 0°; 24 hr); oil; $[\alpha]_D^{25}+36.5°$ (MeOH; *c* 0.16); IR ($CHCl_3$) 1685 cm^{-1} (unsaturated aldehyde); 1736, 1720 and 1656 cm^{-1} corresponding to the three ester groups; and hydroxyl bands at 3440 cm^{-1}.

The 270 MHz ^1H NMR spectrum of **7** resembled that of **5** except for the disappearance of the phenolic proton signal at δ11.64 and the appearance of two multiplets at 7.4–7.6 (5H) and at 8.1–8.2 (5H). The downfield shift of the aromatic protons (6.67, *J* = 2.2, H-6′; 6.51, *J* = 2.2, H-4′) and the methylene protons at C-15 (4.29,(AB(*q*), 2H, CH_2OH) pinpointed the benzoyl group position relative to **5**. The ^{13}C NMR spectrum ($CDCl_3$) supported the proposed structure (**7**). The relative configuration of the methylene group at position C-15 of **7** and hence of **5** itself, was established by NOE experiments (Fig. 1). A large NOE enhancement of the vinylic proton (H-3) on irradiation of the methyl signal (Me-14) revealed the α-postion of Me-14. The similarity of this compound to the illudin S metabolite from *Clitocybe illudens* [5] suggests that biosynthetically the group of sesquiterpenes from *A. mellea* may arise from si, *trans*, *trans*-farnesyl pyrophosphate cyclisation.

5′-*O*-Methylmelledonal (**6**) mp 170–171°; $[\alpha]_D^{25}+64.7°$ (MeOH; *c* 0.34), had a molecular formula of $C_{24}H_{30}O_8$ established by elemental analysis and FAB MS ($[MH]^+$ *m/z* = 447). The IR spectrum (KBr) with hydroxyl (3440 cm^{-1}) and ester (1640 cm^{-1}) bands, and the CIMS $[M-H_2O]^+$ peak at *m/z* 429 with a base peak at *m/z* 165 (everninate group [4]) suggested an everninate version of melledonal (**3**). The proposed structure **6** was consistent with the 67.8 MHz ^{13}C NMR spectrum which was identical to that of melledonal **3** [3] apart from an extra methoxyl signal at δ55.24. A methoxyl signal 3.78 (*s*, 3H) in the 270 MHz ^1H NMR spectrum of **6** which otherwise was identical to that of **3** [3] confirmed the identity of **6** as 5′-*O*-methylmelledonal.



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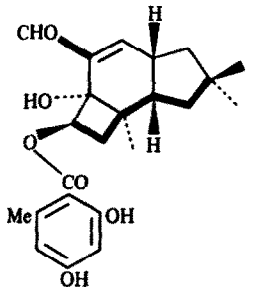

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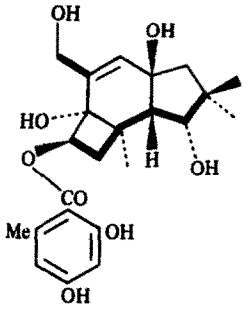

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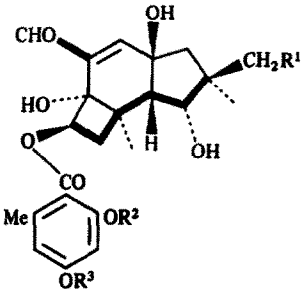

3 R^1, R^2, R^3 = H
5 R^1 = OH, R^2 = H, R^3 = Me
6 R^1, R^2 = H, R^3 = Me
7 R^1 = OBz, R^2 = Bz, R^3 = Me

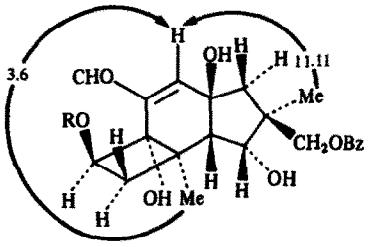

Fig. 1. NOE (%→) Values of compound 7. R = $-COC_6H_2-(OMe)(OBz)Me$.

EXPERIMENTAL

Mp are uncorr. ^{1}H NMR (270 MHz) and ^{13}C NMR (67.8 MHz) spectral determinations, as well as NOE and decoupling experiments, were carried out in $CDCl_3$ with TMS as int. standard. IR spectra were recorded in KBr while UV spectra and $[\alpha]_D$ measurements were obtained in MeOH. Merck Kieselgel 60 (70–230 mesh), Woelm dry silica TSC 04526 and Sephadex LH-20 were used as stationary phases for column chromatography.

Isolation and purification of metabolites. A strain of *A. mellea* (Vahl ex Fr) Kummer (CBS: 120.59) was grown on 5% Difco potato dextrose broth in 10 × 2.5 l penicillin flasks containing a trace of EtOH to promote mycelial growth. After 5 weeks growth at 25° mycelia were harvested by filtration and the culture broth (10 l.) was extracted 2 × with Merck *n*-hexane, then × 3 with EtOAc. The extract was dried ($MgSO_4$) and evapd to give a brown oily residue (240 mg). This was chromatographed on Sephadex LH-20 using gradient elution [CH_2Cl_2–*n*-hexane, 4:1; CH_2Cl_2–Me_2CO 3:2; CH_2Cl_2–Me_2CO 1:4]. Two crude fractions were obtained.

Fraction A (55 mg) was chromatographed on a column of silica gel using gradient elution with $CHCl_3$–EtOAc–MeOH [50:10:1 → 50:10:3] followed by gel chromatography (Sephadex LH-20 (MeOH)) to yield 5′-*O*-methylmelledonal (4) (18 mg). (Found: C, 64.83; H, 7.33. $C_{24}H_{30}O_8$ requires: C, 64.57; H, 6.72%).

Fraction B (120 mg) was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O [30:1.6:0.1] followed by gradient elution on silica gel using $CHCl_3$–MeOH [98:2 → 95:5]. The resulting solid (20 mg) was further purified using Sephadex LH-20 gradient elution (CH_2Cl_2–hexane, 4:1 → CH_2Cl_2–Me_2CO, 3:2 → CH_2Cl_2–Me_2CO, 1:4) followed by a short column of Sephadex LH-20 (MeOH) to give 15-hydroxy-5′-*O*-methylmelledonal (5) (11 mg). (Found: C, 62.70; H, 7.94. $C_{24}H_{30}O_9$ requires: C, 62.34; H, 6.49%).

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REFERENCES

1. Donnelly, D., Sanada, S., O'Reilly, J., Polonsky, J., Prangé, T. and Pascard, C. (1982) *J. Chem. Soc. Chem. Commun.* 135.
2. Midland, S. L., Izac, R. R., Wing, R. M., Zaki, A. I., Munnecke, D. E. and Sims, J. J. (1982) *Tetrahedvon Letters*, 23, 2515.
3. Donnelly, D., Coveney, D. J. and Polonsky, J. (1985) *Tetrahedron Letters* 26, 5343.
4. Donnelly, D., Coveney, D. J., Fukuda, N. and Polonsky, J. (1986) *J. Nat. Prod.* 49, 111.
5. Cane, D. E. and Nachbar, R. B. (1978) *J. Am. Chem. Soc.* 100, 3208.

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TANAVULGAROL, AN OXYGENATED SESQUITERPENE WITH AN UNCOMMON SKELETON FROM *TANACETUM VULGARE*

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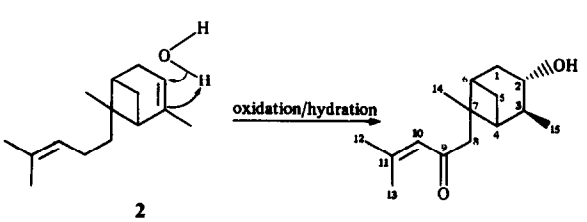
Key Word Index—*Tanacetum vulgare*; Asteraceae; sesquiterpene; tanavulgarol.

Abstract—The reinvestigation of a fraction of *Tanacetum vulgare* extract afforded an oxygenated bergamotane derivative. The structure was elucidated by spectroscopic methods. The biogenetic origin of the compound is discussed.

In continuation of our investigation of *T. vulgare* L. [1] we report the isolation and characterization of a new sesquiterpenoid, tanavulgarol, with the bergamotane skeleton.

The ^{1}H NMR spectrum of **1** showed an AB system [J_{AB} = 18 Hz] at δ2.60 and 2.70. A downfield broad singlet at 5.70 and two singlets at 2.00 and 1.70 together with the above AB system clearly indicated the presence of a $CH_2COCH{=}C(Me)_2$ chain. A double triplet at 4.10 showed that the molecule contained of hydroxy moiety. Furthermore its mass spectrum showed $[M]^+$ at *m/z* 236 ($C_{15}H_{24}O_2$) and $[M-H_2O]^+$ at *m/z* 218 ($C_{15}H_{22}O$), which indicated that the molecule is a sesquiterpene alcohol. Nonavailability of further signals for vinylic protons and the presence of a singlet at δ0.90 and a doublet (J = 7 Hz) at 0.88 in its ^{1}H NMR spectrum suggested it to be a bicyclic sesquiterpene consisting of a 4-methylpent-3-en-2-one chain. The literature [2, 3] showed that the bergamotenes (**2**) have been isolated with similar structure. The differences in the ^{1}H NMR spectrum were (i) a hydroxy group adjacent to a secondary methyl in place of a vinylic proton and a vinylic methyl and (ii) a conjugated ketone which shifted the original vinylic proton further down field to δ5.70. The irradiation of the multiplet at δ2.13 collapsed the doublet at 0.88 into a singlet and the double triplet at 4.10 into a triplet. This suggested that the cyclic double bond of α-bergamotene has been hydrated to yield **1**.

Brown *et al.* [4, 5] have shown through a series of reactions on cyclic olefins that hydration of such double bonds proceeds via the anti-Markownikoff's rule and observed that the reaction proceeds stereospecifically to add the elements of water in a *cis*-configuration from the less hindered side of the double bond. This generalization helped us in establishing the stereochemistry of the hydroxy group as α, which was supported by its coupling constants in the ^{1}H NMR spectrum [6]. Though we could not establish its absolute configuration, these data along with the IR and UV spectra were in complete agreement with the proposed structure of tanavulgarol (**1**). A scheme representing the biogenesis of this skeleton is depicted in Scheme 1. The earlier isolated compound α-bergamotene (**2**) has been shown to be formed by enzymatic cyclization and dehydrogenation which after anti-Markownikoff hydration would have yielded **1** [2].



2 **1**

Scheme 1.