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BENZOPYRAN DIMERS FROM *MELICOPE PTELEFOLIA*

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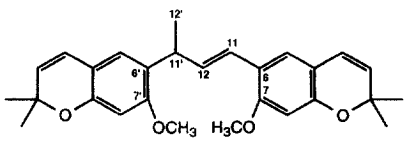
Key Word Index—*Melicope ptelefolia*; Rutaceae; leaves; 2,2-dimethyl-2H-1-benzopyran dimers.

Abstract—The leaves of *Melicopte ptelefolia* afforded two new 2,2-dimethyl-2H-1-benzopyran dimers named 5,5′-dimethoxy-alloagerasanin and melifolin. Their structures were established by NMR spectroscopy, especially NOE and HMBC.

INTRODUCTION

Melicope ptelefolia (Champ. ex Benth.) Hartley [1], described by Ho as *Euodia lepta* (Spreng.) Merr. (= *Evodia lepta* = *E. roxburghiana* Pierre) [2], is a shrub growing in many areas of Vietnam. Its leaves and twigs are used for the treatment of itches and wounds, whereas its root and bark serve as an appetizer, digestive and emmenagogue [3]. *M. ptelefolia* is known to contain alkaloids of the quinoline-, furoquinoline- and pyranoquinoline-types [4–6]. In a previous paper, we described 18 2,2-dimethyl-2H-1-benzopyrans from the leaves of *M. ptelefolia* [7], and very recently some further benzopyrans were published from this plant species [8]. In continuation of this work, we now report on the isolation and structural elucidation of two new 2,2-dimethyl-2H-1-benzopyran dimers, for which the structures have been established as 5,5′-dimethoxy-alloagerasanin (**1**) and melifolin (**2**).

Agerasanin



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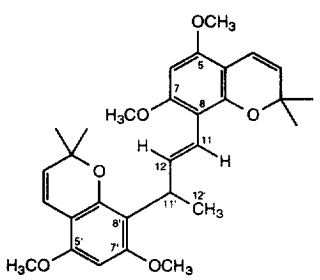

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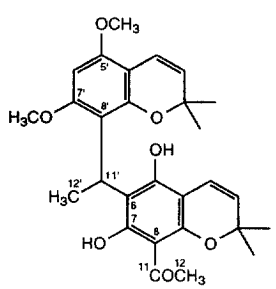

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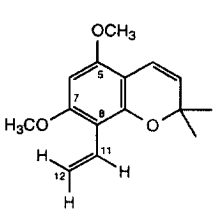

RESULTS AND DISCUSSION

A *n*-hexane extract of leaves of *M. ptelefolia* afforded, besides the previously described 2,2-dimethyl-2H-1-benzopyrans [7], two further compounds **1** and **2**. The molecular mass of compound **1** ($C_{30}H_{36}O_6$, $[M]^+$ *m/z* 492) is exactly twice that of 8-vinyl-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran (**3**), which is also a component of this extract [7]. The NMR spectra (Table 1) are similar to those of **3**, but displays pairs of signals with only small differences in chemical shift (*ca* 0.1–0.5 ppm for carbon and 0.01 ppm difference for proton shift). These facts suggest that **1** is a dimer of **3**. Instead of the vinyl group, the ^{1}H NMR spectrum exhibits signals at δ 6.59) *dd*, J = 16.0 and 1.0 Hz), (*dd*, J = 16.0 and 8.2 ppm), 4.15 (*dqd*, J = 8.2, 7.0 and 1.0 Hz) and 1.40 (*d*, J = 7.0 Hz), which can be combined to give a (E)—CH=CH—CH(CH_3)-bridge

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Table 1. ^1H and ^{13}C NMR spectral data of compound **1** in acetone-d_6 (300/75 MHz)

	δ_C	δ_H J (Hz)	HMBC correlations
2,2′	76.6, 76.4	—	2/2′-Me_2, H-4/4′
3,3′	127.0, 126.8	5.51 *d* (9.9), 5.50 *d* (9.9)	2/2′/Me_2
4,4′	118.0, 117.8	6.57 *d* (9.9), 6.56 *d* (9.9)	
5,5′	155.1, 154.9	—	H-6/6′, OMe
6,6′	89.8, 89.2	6.24 *s*, 6.23 *s*	
7,7′	159.7, 159.5	—	H-6/6′, OMe
8	109.4	—	H-6, H-12
8′	116.1	—	H-6′, H-12′
9,9′	152.8, 152.7	—	H-4/4′
10,10′	105.4, 104.9	—	H-3/3′, H-6/6′
11	118.9	6.59 *dd* (16.0/1.0)	H-11′
11′	35.2	4.15 *dqd* (8.2/7.0/1.0)	H-11, H-12, H-12′
12	137.8	7.02 *dd* (16.0/8.2)	H-11′, H-12′
12′	20.4	1.40 *d* (7.0)	H-11′, H-12
2-Me_2, 2′-Me_2	28.03, 27.99, 27.90, 27.63	1.42 *s*, 1.42 *s*, 1.40 *s*, 1.39 *s*	
OMe	56.2, 55.9, 55.9, 55.9	3.86 *s*, 3.84 *s*, 3.83 *s*, 3.82 *s*	

by analysis of the HH-coupling constants. This connection and the substitution pattern is confirmed by the HMBC data (Table 1). The differentiation of the similar carbons and protons of the two benzopyran moieties was not possible on account of the nearly identical chemical shifts. Compound **1** is similar to agerasanin from *Ageratina arsenii* [9] and thus can be named 5,5′-dimethoxy-alloagerasanin. Compound **1** was identified as a product of 8-(1-hydroxyethyl)-5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran and of **3**, which are also present in the extract [7] and are unstable in $CDCl_3$, which normally contains traces of DCl.

Compound **2** ($C_{28}H_{32}O_7$; $[M]^+$ *m/z* 480), named melifolin, is also a benzopyran dimer, as all benzopyran signals occur twice. Additionally, the ^1H NMR spectrum (Table 2) shows one acetyl singlet (δ 2.67), two methoxyl singlets (δ 3.81 and 3.86), one chelated (δ 14.43) and one non-chelated (δ 8.45) hydroxyl group, one lone aromatic proton (δ 6.07) and a CH—CH_3 group (δ 5.33, *q*, δ 1.68, *d*, J = 7.9 Hz), which is supposed to connect the benzopyran moieties. The location of the substituents follows from the NOE interactions (Fig. 1). The structure of **2** is confirmed by the mass spectral fragmentation which shows the two monomeric parts at *m/z* 260 (corresponding to an acetyldihydroxyvinyl-dimethylbenzopyran) and at *m/z* 220 (corresponding to a dimethoxydimethylbenzopyran). Further evidence for this structure is provided by the carbon shifts. Those of one benzopyran moiety are nearly identical with the corresponding part of **1**, those of the other correspond very well to the carbon shifts of the similar compound 8-acetyl-5,7-dimethoxy-6-isopentenyl-2,2-dimethyl-2H-1-benzopyran [7]. Differences are observed only at the connecting carbons C-6 and C-8′.

The co-occurrence of **3** together with **1** and **2** strongly suggests, that **3** is the biosynthetic precursor of **1** and **2**.

Table 2. ^1H and ^{13}C NMR spectral data of compound **2** in $CDCl_3$ (300/75 MHz)

	δ_C	δ_H J (Hz)
2	77.5	—
3	124.0	5.36 *d* (10.0)
4	116.8	6.60 *d* (10.0)
5	157.9	—
6	111.1[b]	—
7	163.7	—
8	105.2	—
9	155.0	—
10	102.2	—
11	203.2	—
12	33.2	2.67 *s*
2-Me_2	27.8[a]	1.47 *s*
	27.8[a]	1.43 *s*
5-OH	—	8.45 *s*
7-OH	—	14.43 *s*
2′	77.5	—
3′	126.3	5.45 *d* (10.0)
4′	117.4	6.59 *d* (10.0)
5′	154.4	—
6′	89.0	6.07 *s*
7′	158.7	—
8′	112.5[b]	—
9′	151.8	—
10′	104.8	—
11′	23.6	5.33 *q* (7.9)
12′	18.8	1.68 *d* (7.9)
2′-Me_2	28.0[a]	1.50 *s*
	26.6[a]	1.32 *s*
5′-OMe	56.0[c]	3.81 *s*
7′-OMe	55.6[c]	3.86 *s*

[a,b,c] Assignment interchangeable.

The content of acetyl-2,2-dimethyl-2H-1-benzopyrans in *Melicope* species is taxonomically significant, because the occurrence of acetophenone derivatives in the Rutaceae family is very restricted [10].

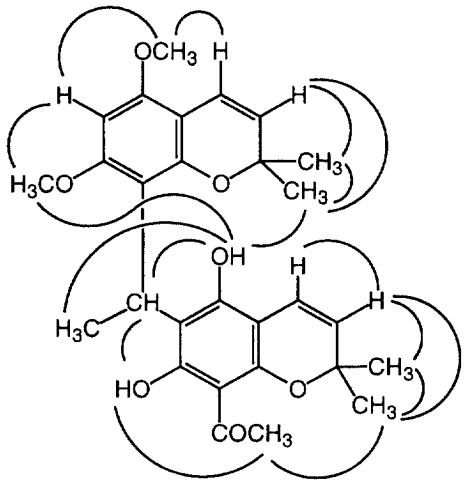

Fig. 1. NOE interactions observed in **2**.

Besides these benzopyrans, several benzodipyrans have been isolated from other *Melicope* species [11]. 2,2-Dimethyl-2H-1-benzopyran dimers are very scarce. Some are knwon from the Asteraceae family, for example the above mentioned agerasanin [9].

EXPERIMENTAL

General. EI-MS: 70 eV; CC: silica gel 60, 70–200 and 230–400 mesh ASTM (Merck); TLC: precoated silica gel plates 60 F_{254} (Merck), detection: UV-light, spray reagent: vanillin in H_2SO_4.

Plant material. Leaves and branches of *Melicope ptelefolia* were collected in Lao cai, North Vietnam, in June 1994 and identifeid by Dr T. D. Dai. A voucher specimen (No. 382) is deposited in the Institute of Ecology and Natural Resources, National Centre for Scientific Research and Technology, Hanoi, Vietnam.

Isolation. Dried leaves (500 g) were extracted ×3 with 80% aq. MeOH at room temp. and the organic solvent removed under red. pres. The aq. residue was extracted ×3 with *n*-hexane giving 17.3 g *n*-hexane extract. This extract was sepd by chromatography on silica gel (200 g, 70–200 mesh) with increasing amounts of EtOAc in *n*-hexane as eluent (2–100% EtOAc, 237 frs, each 20 ml). Frs 89–113 (650 mg) were further purified by chromatography on silica gel (230–400 mesh) using *n*-hexane–EtOAc (7:3) to yield 19 mg of **2**. Frs 114-136 (1469 mg) afforded 21 mg of **1** after purification with *n*-hexane-EtOAc (7:3).

5,5′-Dimethoxy-alloagerasasin (**1**), 1,3,-bis-(5,7-*dimethoxy*-2*H*-1-*benzopyran*-8-*yl*)-(E)-1-*butene*. Amorphous solid, R_f 0.37 (*n*-hexane–EtOAc, 7:3). $[\alpha]_D^{25}$ −0.4° (MeOH, *c* 0.53). UV λ_{max}^{MeOH} nm (log ε): 274 (4.63), 205 (4.49); IR ν_{max}^{KBr} cm^{-1}: 2970, 2934, 2837, 1636, 1596, 1465, 1325, 1253, 1208, 1150–1090, 881, 771; MS *m/z* (rel. int.): 492 $[M]^+$ (32), 477 $[M-Me]^+$ (36), 461 (5), 259 (34), 247 (100), 245 (18), 233 (12), 231 (18), 205 (6), 85 (8); HRMS 492.2483 $[M]^+$ ($C_{30}H_{36}O_6$ requires 492.2512).

Melifolin (**2**), 6-[1-(5,7-*Dimethoxy*-2,2-*dimethyl*-2*H*-1-*benzopyran*-8-*yl*)*ethyl*]-8-*acetyl*-5,7-*dihydroxy*-2,2-*dimethyl*-2*H*-1-*benzopyran*. Amorphous solid R_f 0.40 (*n*-hexane–EtOAc, 7:3), $[\alpha]_D^{23}$ +2.0°, (MeOH, *c* 0.37). UV λ_{max}^{MeOH} nm (log ε): 280 (4.56), 229 (4.58); IR ν_{max}^{KBr} cm^{-1}: 3335, 2970, 2929, 2848, 1639, 1602, 1465, 1364, 1263, 1119, 800; MS *m/z* (rel. int.): 480 $[M]^+$ (14), 465 $[M-Me]^+$ (8), 433 (4), 260 [8-acetyl-5,7-dihydroxy-6-vinyl-2,2-dimethyl-2H-1-benzopyran]$^+$ (20), 245 $[260-Me]^+$ (37), 220 [5,7-dimethoxy-2,2-dimethyl-2H-1-benzopyran]$^+$ (75), 205 $[220-Me]^+$ (100); HRMS 480.2131 $[M]^+$ ($C_{28}H_{32}O_7$ requires 480.2148).

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REFERENCES

1. Editorial Committee of the Flora of Taiwan, *Flora of Taiwan*, 2nd edn, Vol. 3. Taipei, Taiwan, 1993, p. 521.
2. Pham-hoang Ho, *Cayco Vietnam, An Illustrated Flora of Vietnam*, Vol. 2, author-publisher, Montreal, 1993, p. 515.
3. Do Tat Loi, *Nhung cay thuoc va vi thuoc Viet Nam* (Glossary of Vietnamese Medicinal plants). Nha xuat ban Khoa hoc va Ky thuat (Science and Technics Publication), Hanoi, Vietnam, 1977, p. 145.
4. Goh, S. H., Chung, V. C. and Jones, D., *Bulletin of the Singapore National Institute of Chemistry*, 1989, **17**, 21.
5. Gunawardana, Y. A. G. P., Cordell, G. A., Ruangrungsi, N., Chomya, S. and Tantivatana, P., *Journal of the Science Society of Thailand*, 1987, **13**, 107.
6. McCormick, J. L., McKee, T. C., Cardellina II, J. H. and Boyd, M. R., *Journal of Natural Products*, 1996, **59**, 469.
7. Kamperdick, C., Van, N. H., Sung, T. V. and Adam, G., *Phytochemistry*, 1997, **45**, 1049.
8. Li, G.-L., Zeng, J.-F., Song, C.-Q. and Zhu, D.-Y., *Phytochemistry*, 1997, **44**, 1175.
9. Fang, N., Yu, S. and Mabry, T. J., *Phytochemistry*, 1988, **27**, 1902.
10. Quader, A., Armstrong, J. A., Gray, A.I., Hartley, T. G. and Waterman, P. G., *Biochemical Systematics and Ecology*, 1991, **19**, 171.
11. Muyard, F., Bissoue, A. N., Bevalot, F., Tillequin, F., Cabalion, P. and Vaquette, J., *Phytochemistry*, 1996, **42**, 1175.