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A 3,4-*SECO*-AMBROSANOLIDE FROM *AMBROSIA ARTEMISIIFOLIA*

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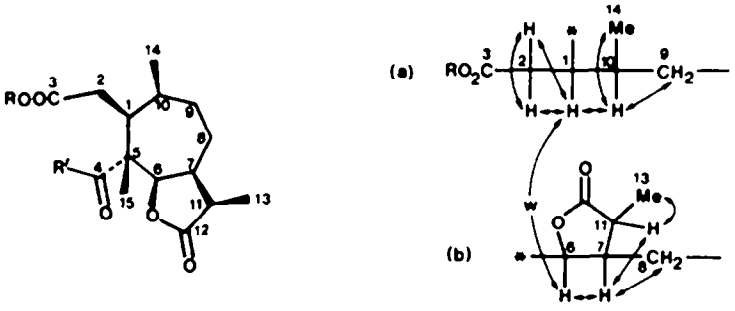
Abstract –A new 3,4-*seco*-ambrosanolide was isolated from *Ambrosia artemisiifolia* and identified by means of spectroscopic evidence (^{1}H and ^{13}C NMR, IR and MS).

A number of sesquiterpene lactones belonging to the pseudoguaianolide (and also 4,5-*seco*-pseudoguaianolide) group have been isolated so far from *Ambrosia artemisiifolia* species of various geographic origins [1–4]. Our previous investigations concerning Yugoslavian species (originating from the locality near Novi Sad) [3, 4] also revealed the same type of γ-lactones, such as 4-hydroxy-3-oxo-pseudoguaian-6,12-olide (**1**), 8α-acetoxy-3-oxo-pseudoguaian-6,12-olide (**2**), psylostachin (**3**) and psylostachin C (**4**). As a continuation of these chemotaxonomic studies, five γ-lactones were isolated from the $CHCl_3$ extract of the whole plant of *Ambrosia artemisiifolia* (collected near Pančevo, Yugoslavia). Whereas four of them were readily assigned (according to the spectral evidence) as the known compounds, such as cumanin (**5**) [1, 5], cumanin diacetate (**6**) [5] and the already mentioned compounds **2** and **4**, the remaining crystalline lactone (**7**) (CIMS: M + H, *m/z* 283, corresponding to the molecular formula of $C_{15}H_{22}O_5$) was shown to be new. This compound exhibited structural features similar to those of ambrosanolides **1** and **2**, i.e. 6,12-γ-lactone moiety (1770 cm^{-1}; δ4.74, *br d*, $J \simeq 5$ Hz, lactonic proton, H-6), two secondary methyls giving rise to three-proton doublets (δ1.11, $J \simeq 7.5$ Hz and δ0.92, $J \simeq 7$ Hz, H_3-13 and H_3-14, respectively) and a tertiary methyl (δ1.30, *s*, H_3-15). The magnitude of the vicinal coupling concerning the lactonic proton was in accordance with the *cis*-fusion of the lactone ring, which is typical for ambrosanolides. The presence of an isolated aldehyde group (1725 cm^{-1}; δ9.58, *s*), as well as a carboxylic function (*ca* 1715, 2400–3500 cm^{-1}; δ10.58 *br s*) indicated a biogenetically plausible structure, that of a 3,4-*seco*-pseudoguaian-6,12-olide (Scheme 1), possibly obtained via an oxidative cleavage of the C-3/C-4 bond in the 4-hydroxy-3-oxoprecursor (**1**), previously identified in the same plant species [4]. This type of 3,4-fragmentation was previously encountered only in the helenanolide series (i.e. pseudoguaian-8,12-olide with 10α-positioned methyl), leading to dilactones, such as vermeerin and greenein [6]. The ^{13}C NMR spectrum of **7** (see Experimental), revealing (in addition to the functionalities quoted so far) three methylenes (C-2, C-8 and C-9), three



7 R = R' = H

8 R = Me, R' = H

9 R = R' = Me

(* = quaternary carbon; ⌇ = coupling)

Scheme 1.

methine groups (C-1, C-7 and C-11) and a quaternery carbon (C-5), which all fit the proposed structure.

Upon treatment with CH_2N_2 (in ether) compound **7** was converted to the corresponding methyl ester (**8**) and, to a smaller extent, into the keto-ester (**9**). After the separation of these compounds (by means of column chromatography) a ^1H NMR (400 MHz) study of the major product **8** was undertaken. Whereas the signals due to H-1, H-2, H-7 and H-11 were superimposed in the spectrum of **8** measured in $CDCl_3$ (Table 1), the application of C_6D_6 as the NMR solvent enabled their resolution and, with the aid of spin decoupling, almost a complete spectral assignment, revealing coupling patterns (Scheme 1, **a** and **b**) fully compatible with the proposed gross structure. At the same time, vicinal couplings concerning H-10 measured in the $^1H\{$Me-14$\}$ NMR spectrum of **8** (in $CDCl_3$), assigned to an axial–axial ($J_{10\alpha,9\beta} \simeq 10$ Hz) and two axial–equatorial couplings ($J_{10\alpha,1\alpha} \simeq J_{10\alpha,9\alpha} \simeq 4$ Hz), indicated a chair (or a skew chair) conformation for the seven-membered ring with *cis*-positioned CH_2COOH and Me-14, occupying quasi-axial (β) and quasi-equatorial (β) positions, respectively. A confirmation of the quasi-axial position of the CH_2COOH group is also obtained by the observance of a long-range *W* coupling between H-1 and H-6, which is typical for 1,3-equatorially positioned protons. The remaining proton from the ring junction (H-7) could be placed (according to the magnitude of $J_{6,7}$) in a *cis* (*gauche*) position with respect to H-6, which is in accordance with a half-chair conformation of the pentacyclic ring. In such a case, the magnitude of $J_{11,7}$ of ≥ 7 Hz, which could be interpreted (according to the well-known Karplus equation) either as a *cis*- or a *trans*-coupling, is more likely to be assigned to the former, corresponding to 11β-orientation of Me-13. This is also supported by the observed considerable diamagnetic shift of H-11 in C_6D_6 (i.e. $\delta_{CDCl_3} - \delta_{C_6D_6} = 0.79$ ppm) which could be explained by a positioning of benzene in a parallel alignment with the lactone ring at the side occupied by H-11, and (according to Dreiding models) it could only be the α-side, since the β-side is protected by the seven-membered ring. The stereochemical assignment of the quaternary centre (C-5), i.e. 5β-Me and 5α-CHO (as shown in Scheme 1), is mostly based on the proposed biogenetical relationship of lactone **7** to the previously detected co-occurring ambrosanolide **1** [4]. It should also be noted that the application of the aromatic NMR solvent altered some of the vicinal couplings in **8** (e.g. those concerning H-6 and H-10, see Table 1), thus indicating conformational changes.

The ^1H NMR spectrum of keto-ester **9** (Table 1), the product obtained by the reaction of **7** with CH_2N_2 (involving both COOH and CHO groups), differed from the spectrum of **8** by the occurrence of a three-proton singlet (δ2.29, MeCO) instead of a low-field signal of CHO and this also fits the proposed structure.

EXPERIMENTAL

Plant material. Ambrosia artemisiifolia L. (specimen No. 250783) was identified and collected by Ž. Joksimović (Botanic Garden, Faculty of Science, Belgrade) in summer 1983, near Pančevo (*ca* 15 km north-east from Belgrade) Yugoslavia.

Isolation procedure. A crude $CHCl_3$ extract (52 g), obtained from the powdered air-dried whole plant (5 kg) using the usual procedure [3, 4], was chromatographed on a silica gel column. The elution was started with C_6H_6 and the polarity of the eluent was gradually increased by addition of Et_2O. The lactones, eluted in the following order (the ratio of C_6H_6–Et_2O is given in parentheses): **2** (9.5:0.5), **6** (9:1), **7** (8.5:1.5), **4** (8.5:1.5) and **5** (4:1), were isolated from the crude fractions by rechromatography and/or crystallization. The identification of the known compounds, i.e. 8α-acetoxy-3-oxo-pseudoguaian-6,12-olide (**2**,

Table 1. ^1H NMR spectral data of compounds **7** (80 MHz), **8** (400 MHz) and **9** (400 MHz) (TMS as internal standard)

H	**7** [$(CD_3)_2CO$]	**8** ($CDCl_3$)	**8** (C_6D_6)	**9** ($CDCl_3$)
1	2.5–2.9 *m*		2.61 *dt*	
2A	2.72 *dd*	2.7 2.9 *m*	2.72 *dd*	2.8–2.9 *m*
2B	2.32 *dd*		2.18 *dd*	
4	9.58 *s*	9.49 *s*	8.88 *s*	2.29 *s* (MeCO)
6	4.74 *d* (*br*)	4.71 *d* (*br*)	4.82 *dd*	4.72 *d* (*br*)
7	2.5–2.9 *m*	~ 2.85 *m*	2.26 *ddt*	2.95 *ddt*
8α			1.09 *m* (1H),	
8β	1.2–1.6 *m*	1.4–1.6 *m*	0.8–1.0 *m*	1.15 1.5 *m*
9α			(3H)	
9β		1.25 *m*		
10	1.69 *m*	1.64 *m*	1.22 *m*	1.58 *m*
11	3.02 *qui*	2.87 *qui*	2.08 *qui*	2.8–2.9 *m*
13	1.11 *d*	1.16 *d*	0.84 *d*	1.14 *d*
14	0.92 *d*	0.91 *d*	0.66 *d*	0.87 *d*
15	1.30 *s*	1.32 *s*	1.01 *s*	1.41 *s*
COOH(Me)	10.58 *s* (*br*)	3.70 *s* (Me)	3.32 *s* (Me)	3.69 *s* (Me)

J (Hz): in **7**: 1,2A = 4; 1,2B = 6.5; 2A,2B = −18; 6,7 = 5; 7,11 = 11,13 = 7.5; 10,14 = 7; in **8** ($CDCl_3$): 1,10 = 10,9α = 4; 10,9β = 10; 6,7 = 4.5; 7,11 = 11,13 = 7; 10,14 = 7; in **8** (C_6D_6): 1,2A = 4; 1,2B = 6; 2A,2B = −18; 1,10 = 10,9α = 4; 10,9β = 8.5; 6,7 = 5.5; 1,6 ≥ 0.6; 7,8α = 7; 7,8β = 5; 7,11 = 11,13 = 7.5; 10,14 = 7; in **9**: 6,7 = 5; 7,8β = 5; 7,8α = 6 or 8; 7,11 = 6 or 8; 10,14 = 11,13 = 7.

~ 170 mg), cumanin diacetate (6, ~ 20 mg), psylostachin C (4, ~ 30 mg) and cumanin (5, ~ 1.3 g), is based on the identity of their spectral data (^{1}H NMR and IR) to the published ones [1, 3, 5]. A specimen of cumanin (5) was also converted to its diacetate (6) by acetylation with Ac_2O in pyridine at room temp. (for 24 hr).

4-Oxo-3,4-*seco*-ambrosan-6,12-olide-3-oic acid (7) was isolated from the crude fraction by crystallization from Me_2CO–petrol; mp (uncorr) 171.5–176°, $[\alpha]_D^{20}$ −0.88° and $[\alpha]_{365}^{20}$ −42.30° (Me_2CO, *c* 0.910); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone C=O), 1725 (aldehyde C=O), 2400–3500, 1715 (COOH); CIMS (iso-butane), 70 eV, *m/z* (rel. int.): 283 $[M+H]^+$ (12), 281 $[M-H]^+$ (2.5), 265 $[M+H-H_2O]^+$ (100), 263 $[M-H-H_2O]^+$ (5), 247 $[M+H-2\times H_2O]^+$ (4.5), 237 $[M+H-H_2O-CO]^+$ (9), 235 $[M-H-H_2O-CO]^+$ (4.5), 219 $[M+H-2\times H_2O-CO]^+$ (8.5); ^{13}C NMR (50 MHz, C_5D_5N + TMS): δ10.5 (*q*, C-13), 21.0 (*q*), 22.0 (*q*), 23.4 (*t*), 29.4 (*t*), 30.7 (*t*), 37.1 (*d*), 40.6 (*d*), 41.6 (*d*), 43.5 (*d*), 55.9 (*s*, C-5), 85.3 (*d*, C-6), 176.7 (*s*, C-3), 178.4 (*s*, C-12), 203.7 (*d*, C-4); ^{1}H NMR: see Table 1.

4-Oxo-3,4-*seco*-ambrosan-6,12-olide-3-oic acid methyl ester (8) was obtained as a main product by reaction of 7 with CH_2N_2 in Et_2O at the room temp. (overnight). Silica gel column chromatography (C_6H_6–EtAc, 97:3) afforded the crystalline ester 8, mp (uncorr) 102–105°, $[\alpha]_D^{20}$ −0.91° and $[\alpha]_{365}^{20}$ −9.09° ($CHCl_3$, *c* 0.220); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone C=O), 1730 (ester + aldehyde C=O); EIMS (probe) 70 eV, *m/z* (rel. int.): 296 $[M]^+$ (3.5), 278 $[M-H_2O]^+$ (3.5), 267 $[M-CHO]^+$ (10), 265 $[M-OCH_3]^+$ (13), 237 $[M-CO_2CH_3]^+$ (13.5), 223 $[M-C_3H_5O_2]^+$ (19.5), 222 $[M-C_3H_6O_2]^+$ (21), 55 (100), 43 (71), 41 (98); ^{1}H NMR: see Table 1. 4-Methyl-4-oxo-3,4-*seco*-ambrosan-6,12-olide-3-oic acid methyl ester (9) was obtained as a byproduct in the reaction of 7 with CH_2N_2. The purified crystalline compound 9 (eluted after the main product 8, see above), mp (uncorr) 132–134.5°, was identified by comparison of its ^{1}H NMR data to those of compound 8 (Table 1).

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