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GRANILIN AND IVASPERIN FROM *AMBROSIA POLYSTACHYA*. ^{13}C-NMR SPECTRA OF HYDROXYLATED ISOALANTONES*

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Key Word Index—*Ambrosia polystachya*; Compositae; eudesmanolides; sesquiterpene lactones; ^{13}C-NMR spectra.

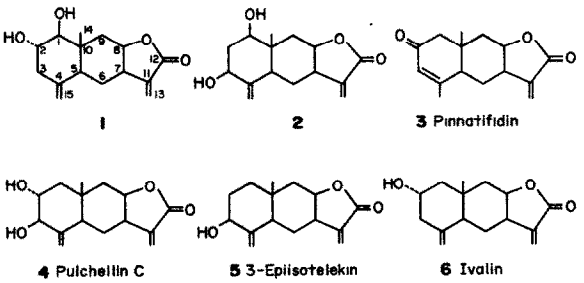
Abstract—The eudesmanolides granilin and ivasperin were isolated from *Ambrosia polystachya* DC. ^{13}C-NMR spectra are reported for these and other naturally-occurring ring A-hydroxylated isoalantolactones.

INTRODUCTION

Since the structure elucidation [1–3] of ambrosin and parthenin from *Ambrosia maritima* L. and *Parthenium hysterophorus* L., respectively, a great deal of attention has been paid to the distribution of sesquiterpene lactones in *Ambrosia* and related genera as a possible means of clarifying evolutionary relationships [4]. In the following we report on the lactone constituents of *Ambrosia polystachya* DC., a species found in South Central Brazil [5] which has not been investigated previously.

RESULTS AND DISCUSSION

One of the lactone constituents was readily identified as the hydroxylated isoalantolactone derivative ivasperin (1) which has been found previously in *Iva asperifolia* Less and *I. texensis* Jackson [6]. A second lactone presented somewhat greater difficulties. The physical and chemical properties, mp 197–199°, UV λ_{max} 210 nm, IR bands at 3280, 1760, 1663, and 1648 cm^{-1}, $[\alpha]_D \sim 110°$, formation of a diacetate, resembled those of granilin, one of several lactones isolated from *Inula grandis* Schrenk, to which formula **2**, devoid of stereochemistry, had been assigned by the Russian discoverers [7]. Since all signals of our substance were beautifully separated in the ^{1}H-NMR spectrum at 270 MHz, spin-decoupling experiments which will not be detailed here and determination of the coupling constants listed in the Experimental Section showed that it had indeed the gross structure of granilin and permitted deduction of the stereochemistry shown in **2**. After completion of our work, another report on the isolation of granilin from *Carpesium abrotanoides* L. (Inuleae) and the determination of its stereochemistry by NMR spectrometry appeared [8]. The mp of our material is higher than that reported by the Japanese workers, but identical with that of the Russian group.



Assignment of a structure to granilin was also made possible by comparing the ^{13}C-NMR spectrum of granilin with the spectra of ivasperin (**1**) and a number of other ring A-oxygenated alantolactones **3–6** isolated by us earlier. The carbon frequencies are listed in Table 1;

Table 1. ^{13}C-NMR spectra of alantolactones*

Carbon atom	Compound 1†	2‡	3†	4‡	5†	6†
1	85.2*d*	73,4*d*	54.2*t*	49.2*t*	40.0*t*§	51.0*t*
2	72.2*d*	38.8*t*	196.9	73.2*d*	32.1*t*	66.9*d*
3	44.3*t*	74.2*d*	127.7*d*	79.5*d*§	73.0*d*	46.3*t*
4	147.5	149.2	159.6	150.1	151.0	146.1
5	45.6*d*	43.2*d*	44.9*d*	45.2*d*	44.6*d*	45.6*d*
6	28.3*t*	25.9*t*	26.9*t*	28.6*t*	27.5*t*	27.3*t*
7	41.4*d*	39.4*d*	40.8*d*	41.2*d*	40.3*d*	40.5*d*
8	79.0*d*	76.7*d*	75.6*d*	78.5*d*§	76.7*d*	76.6*d*
9	39.3*t*	33.0*t*	40.6*t*	41.6*t*	41.0*t*§	41.2*t*
10	39.5	39.1	35.7	34.5	34.0	33.9
11	144.3	141.4	141.6	143.7	142.0	142.1
12	172.9	169.6	169.6	172.3	170.5	170.3
13	121.3*t*	119.0*t*	120.6*t*	120.9*t*	120.3*t*	120.1*t*
14	13.3*q*	16.8*q*	18.3*q*	19.0*q*	17.7*q*	18.7*q*
15	109.9*t*	108.8*t*	21.5*q*	106.1*t*	103.5*t*	108.9*t*

* Run on Bruker HX-270 instrument. Unmarked signals are singlets. † Run in $CDCl_3$. ‡ Run in MeOH. § Assignments may be interchanged.

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all assignments for **1** and **2** are based on single frequency off-resonance decoupling experiments except for the C-4, C-10, C-11 and C-13 singlets, which offer no ambiguity. Assignments for **3–6** are based on predicted chemical shifts, internal consistency and, in case of ambiguity (e.g. C-5 and C-7 of **3** and **4** etc.), on single frequency off-resonance decoupling experiments. Inspection of Table 1 reveals that the locus of hydroxyl groups in new alantolactone derivatives is theoretically assignable by using chemical shift parameters, the only anomaly being the abnormal high-field shift of the C-9 methylene of **2** whose spectrum was, however, run in methanol instead of chloroform.

A. polystachya belongs to a group of five South American ragweeds which possess characters believed to be primitive for the genus and may be derived from species which migrated to South America during an early phase of ragweed phylogeny [5]. Among these, *A. polystachya* is unique in its distribution (South Central Brazil vs the inter-andean regions of North-western South America) and in possessing reduced, spineless one-flowered fruiting involucres. The latter are character expressions which are regarded [5] as representative for more advanced species. It has also been suggested [5] that *A. polystachya* has evolved directly from *A. arborescens* Mill. with which it has great similarity in other respects. If this is so, it is not clear how this fits in with the isolation [9] from *A. arborescens* of ambrosanolides and seco-ambrosanolides and from *A. polystachya* of eudesmanolides of the isoalantolactone type. While the eudesmanolides from *A. polystachya* may be considered more "primitive" in the chemical sense than the ambrosanolides and seco-ambrosanolides from *A. arborescens* [4] and the feeling that loss of biosynthetic capacity found in the more primitive members of a taxon, as evidenced by disappearance of compounds lying farther along on the biogenetic pathway, is an advanced character seems to be gaining ground [10], the two classes of compounds lie on different branches of the presumed biogenetic path. The situation however, is somewhat reminiscent of that prevailing in *A. confertiflora* [4b], one of whose four chemical races is of the germacradienolide–eudesmanolide type, while the other three produce germacradienolides, ambrosanolides and modified ambrosanolides, respectively. Sampling of additional populations and of *A. polystachya* and *A. arborescens* and chemical study of the remaining three members of the primitive South American group, *A. artemisioides* [11], *A. pannona* and *A. parviflora*, would clearly be highly desirable.

EXPERIMENTAL

Experimental details have been specified in previous papers. *Extraction of* Ambrosia polystachya. Above-ground parts of the plant, (wt 9 kg) collected by Mr. Hermógenes de Freitas Leitão Filho in February 1974 along the Via Anhanguera, Cordeirópolis, São Paulo State, Brasil, were extracted with EtOH and worked up in the usual manner [12]. Crude gum (wt 20 g) was chromatographed over 570 g of alumina (Merck), 150 ml fractions being collected in the following order: Fr 1–9 (Bz), 10–20 (Bz–EtOAc 3:1), 21–31 (Bz–EtOAc 2:1), 35–39 (Bz–EtOAc 1:1), 40–44 (EtOAc), 45–49 (EtOAc–EtOH 2:1), 50–53 (EtOAc–EtOH 1:1), 54–60 (EtOH). Solid material from fractions 20–40 exhibited one major spot on TLC and was combined, wt 0.4 g. Recrystallization from $CHCl_3$ afforded granilin (**2**), mp 197–199°, UV (EtOH) λ_{max} 210 nm, (ϵ 9240), $[\alpha]_D^{22}$ + 106.8° ($CHCl_3$), CD curve $[\theta]_{256}$ − 7030, IR bands at 3280, 1760, 1663 and 1648 cm^{-1}, significant peaks in the high resolution MS at *m/e* (composition, %) 264 (M^+, $C_{15}H_{20}O_4$, 16), 246 ($C_{15}H_{18}O_3$, 47), 228 ($C_{15}H_{16}O_2$, 60.2), 195 ($C_{14}H_{11}O$, 94.5), 157 ($C_{12}H_{13}$, 33), 143 (C_{11}, 52.5), 99 ($C_5H_7O_2$, 100), 91 (C_7H_2, 53.8). (Calc. for $C_{15}H_{20}O_4$: C, 68.12; H, 7.53; O, 24.40; MW 264.1361. Found: C, 68.16; H, 7.63; O, 24.21%; MW (MS), 264.1375). The 270 MHz NMR spectrum ($CDCl_3$) exhibited signals at 6.14*d* (1.0) and 5.62*d* (0.9, H-13a and H-13b), 5.06*br* (2.5, 1.5, H-15a), 4.70*br* (2.5, 1.0, H-15b), 4.63*td* (5.0, 2.0, H-8), 4.41*m* (3, 2.5, H-3), 3.41*br* (3, 2.5, H-1), 3.01 *m* (12, 7, 5, 1.0, 0.9, H-7), 2.72*dbr* (13, 2.5, 1, 0.9, H-5), AB system at 2.45*dd* (18, 5, H-9a) and 1.88*dd* (18, 2, H-9b), AB system centered at 2.02 (H-2a and H-2b, each *dt* − 17, 3 Hz), AB system at 1.77 *ddd* (14, 7, 2.5, H-6a) and 1.44 *ddd* (14, 13, 12, H-6b), 0.79 (H-14). Diacetate was prepared in the usual manner and could not be induced to crystallize, IR bands at 1760, 1740, 1660 and 1640 cm^{-1}, NMR signals (270 MHz, $CDCl_3$) at 6.16*d* (1.0) and 5.62*d* (0.8, H-13a and H-13b), 5.24*br* (1.5) and 4.84*br* (1.0, H-15a and H-15b), 5.37*m* (3, 2.5, H-3), 4.63*m* (3, 2.5, H-1) partially superimposed on 4.58*td* (5, 2, H-8), 3.05*m* (12, 7, 5, 1, 0.8, H-7), 2.70*m* (13.5, 2.5, 1.5, 1.0, H-5), AB system at 2.50*dd* (18, 5 and 1.91*dd* (18, 2.5, H-9a and H-9b), 2.09 and 2.00 (two acetates) AB system centered at 2.08 (H-2a and H-2b, each *dt* − 17, 2.5 Hz), AB system at 1.80*m* and 1.42*m* (H-ba and H-6b), 0.90 (H-14). The solid material from fractions 46–48 was combined, wt. 0.7 g. Purification by preparative TLC on Si gel (EtOAc–MeOH 5:1) and recrystallization from EtOAc furnished ivasperin, (**1**), mp 157–159°, $[\alpha]_D^{22}$ + 121° ($CHCl_3$), NMR signals (270 MHz, $CDCl_3$) at 6.16*d* (1.0) and 5.62*d* (0.8, H-13a and H-13b), 4.93 *br* and 4.61 *br* (H-14a and H-14b), 4.56*td* (5, 2, H-8), AB system at 3.57*m* (H-2) and 3.12*d* (9, H-1), 2.97*m* (H-7), AB system centered at 1.84 (H-9a and H-9b), AB system centered at 1.46 (H-6a and H-6b), 0.80 (H-14). The low resolution MS exhibited significant peaks at *m/e* 264 (M^+), 246 ($M \pm H_2O$), 228 ($M\text{-}2H_2O$) and 218 ($M^+ - 2H_2O - CO$). There was no mp depression on admixture of an authentic sample of ivasperin (mp raised to 157–159° by further recrystallization), IR and 270 MHz NMR spectra were superimposable.

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