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The structure of amoorastatone and the cytotoxic limonoid 12-hydroxyamoorastatin[1]

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Summary. 2 new limonoid-type terpenes have been isolated from an aqueous extract of seeds produced by the Eastern Himalayan (India) plant *Aphanamixis grandifolia* Bl. By interpreting principally mass spectral and nuclear magnetic resonance data, the structures of 12-hydroxyamoorastatin (**2b**) and amoorastatone (**3**) were elucidated. Unequivocal evidence for the 12-hydroxyamoorastatin structural assignment was obtained by chemical conversion to sendanin (**4**). Amoorastatin derivative **2b** was found to significantly inhibit growth of the murine P388 lymphocytic leukemia cell lines but amoorastatone in the same system was inactive. In a comparative biological study, sendanin (**4**) and anthothecol (**7**) were also found significantly to inhibit growth of the P388 cell line, while rohitukin (**8**) and limonin (**9**) were found to be inactive.

Seeds produced by the Himalayan Meliaceae species *Aphanamixis grandifolia* Bl. have been shown to contain 2 new and highly cytotoxic limonoids assigned structures **1** (aphanastatin)[2] and **2a** (amoorastatin)[3]. Both substances were found strongly to inhibit growth of the murine P388 lymphocytic leukemia[4]. While further developing the isolation of amoorastatin (**2a**), it became possible to isolate 2 companion constituents designated 12-hydroxyamoorastatin (**2b**) and amoorastatone (**3**) that substantially contribute to our knowledge of structure/activity relationships in this new area of limonoid cytotoxic agents. Both of these new limonoids were assigned structures based principally upon spectral evidence with the X-ray crystal structures of aphanastatin (**1**) and amoorastatin (**2a**) serving as established references.

The pure 28 R epimer[5] of 12-hydroxyamoorastatin (**2b**) was obtained as crystals (from acetone) decomposing at 243-245 °C $[\alpha]_D^{22}$ −49.3 °C (c, 0.75, methanol); CD in dioxan, Δ^{ε} −2.52 (310 nm); IR (KBr): 1730 (sh) and 1720 (br) cm^{-1}. The high resolution mass spectrum led to molecular formula ($C_{28}H_{36}O_{10}$ with M^+, m/e 532.2288) and fragmentation ions at m/e 514.2170 (M^+-18), 496.2084 (M^+-36), 472.2103 (M^+-60), 454.1975 ($M^+-60-18$), 436.1872 ($M^+-60-36$) and significant ions at m/e 163.0765 ($C_{10}H_{11}O_2$) and 107.0499 (C_7H_7O). Comparison of the 250 MHz, ^{1}H-NMR spectra obtained from amoorastatin (**2a**) with that of the new limonoid **2b** (Table 1)[6] indicated that the latter possesses an additional hydroxy group at C-12. The small structural modification was further substantiated by inspecting the ^{13}C-NMR which showed in addition to the C-15 resonance (doublet at 59.1) 4-oxygen-bearing

Table 1. Proton magnetic resonance spectra at 250 MHz of 12-hydroxyamoorastatin (**2b**) and amoorastatone (**3**) in deuteriochloroform solution containing approximately 20% pyridine-d_5 and of 12,28-Di-*O*-acetyl 12-hydroxyamoorastatin (**4**) in deuteriochloroform (δ in ppm, J in Hz)[6]

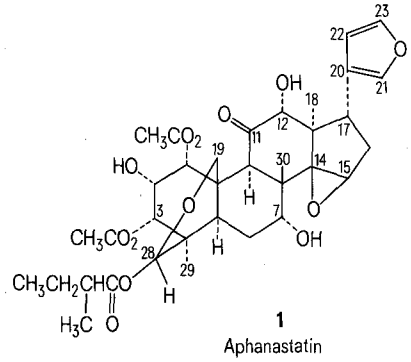
Proton position	**2b**	**3**	**4**
H-1	4.48 d J = 4	4.17 d J = 3.8	4.27 d J = 4.2
H-2	~2.90 m	2.92 t J = 3.8	2.94 m
		3.12 t J = 3.8	2.84 t J = 4.3
H-3	5.47 d J = 4	5.55 d J = 3.8	5.27*
H-7	3.66 br, s	3.72 s	3.68 br, s
H-9	4.10 s	4.08 s	4.61 s
H-12	4.70 s	2.35 AB q J = 17	5.27*
		2.56	
H-14		3.50 s	
H-15	3.78 s		3.76 s
H-16		2.54 d (2 H)	
		J = 10	
H-17	~2.90 m	3.30 t J = 10	2.77 m
H-19	4.31 AB, q	4.26 AB, q	4.32 s (2 H)
	J = 12	J = 13	
	4.41	4.68	
H-21	7.20 s	7.30	7.13 s
H-22	6.57 s	6.28 s	6.15 d J = 1.2
H-23	7.35 s	7.38 s	7.33 d J = 1.2
H-28	4.96 s	5.05 s	5.79 s
Methyl	0.89	0.94	0.83
	1.14	0.94	1.16
	1.26	1.07	1.32
O-acetyl	1.94	2.01	1.98
			2.11
			2.12

* H-3 and H-12 are superimposed.

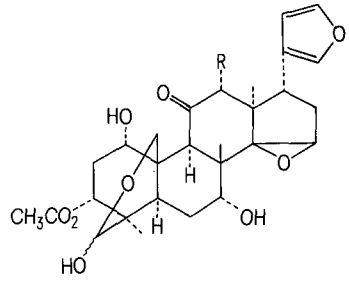
methine carbons (δ 78.63, 74.1, 70.3 and 69.8, each d). As with the closely related hemiacetal **2a**, 12-hydroxyamoorastatin (**2b**) exists in solution as a C-28 epimeric mixture. The hemiacetal isomers were easily recognized by TLC and by the appearance of double ^{13}C signals for C-28 (96.2 and 95.9) and for a few other carbons. Structure **2b** for 12-hydroxyamoorastatin was unequivocally confirmed by chemical correlation with sendanin (**4**)[7] which is an acetyl derivative of the naturally occurring limonoid (**2c**)[8]. Mild acetylation of tetraol acetate **2b** (acetic anhydride/pyridine, 2 h at room temperature) yielded (after chromatographic purification and crystallization from ethyl acetate) a triacetate identical with authentic sendanin (**4**) by m.p., TLC, mass spectra and 250 MHz ^1H-NMR (see table 1).

Amoorastatone (**3**) was found to decompose at 279–281 °C; $[\alpha]_D^{22}$ −68.2 °C (c, 1.025 in methanol), CD (in dioxane), Δ^e −2.87 (295 nm), −3.10 (306 nm) and −2.38 (315 nm); IR (KBr), 1702, 1736 and 1749 cm^{-1}. The molecular formula $C_{28}H_{36}O_9$ (M^+ at m/e 516.2325) of amoorastatone was found isomeric with that of amoorastatin (**2a**) and the electron-impact-induced fragmentation also showed ions corresponding to the loss of acetic acid (1 mole), water (3 moles) and a base peak at m/e 163.0762 ($C_{10}H_{11}O_2$). The latter peak probably arises by a McLafferty rearrangement (followed by cleavage of the C-12, C-13 bond) and provides evidence for a C-15 rather than C-16 oxo group. Comparison of the 250 MHz ^1H-NMR spectra of amoorastatin (**2a**) and amoorastatone (**3**, table 1) with the ^{13}C-NMR spectral data (see table 2) revealed that the 14, 15 epoxide had been replaced by the product of rearrangement, namely a 15-oxo system. Otherwise the substitution pattern of rings A and B were found the same in both amoorastatin and amoorastatone. The C-28 carbon resonances in the proton decoupled ^{13}C-NMR spectrum of amoorastatone (**3**) were assigned utilizing the residual splitting in the off-resonance decoupled spectrum, resonance positions already in the literature[9], and comparison with the spectrum of sendanin (**4**)[7]. Application of the noise off-resonance decoupling technique (NORD)[10] confirmed the presence of only four non-protonated sp^3 carbon atoms and these corresponded to ^{13}C chemical shifts at 40.3, 42.2, 44.5 and 45.7. Finally, the circular dichroism data appeared more consistent with the 14α-configuration assigned[11,12].

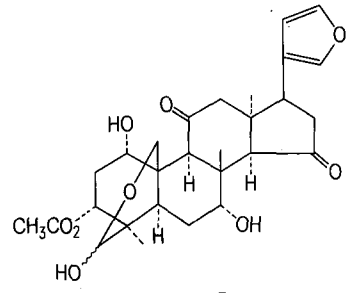
The very substantial P388 cell growth inhibition (ED_{50}= 0.002 µg/ml) exhibited by 12α-hydroxyamoorastatin was between that shown by aphanastatin (**1**) and amoorastatin (**2a**) while amoorastatone (**3**) was considered marginally inactive (ED_{50}= 30 µg/ml). Apparently the 14, 15β epoxide is a very definite requirement for inhibition of neoplastic (P388) cell growth and is reminiscent of analogous structural modification and loss of activity observed with toad poison constituents of the bufadienolide type (compare **5** and **6**)[13].



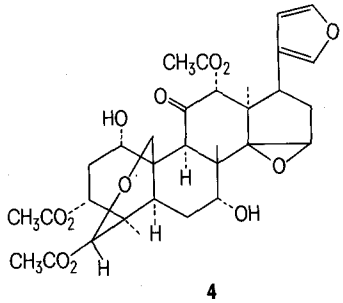
1 Aphanastatin



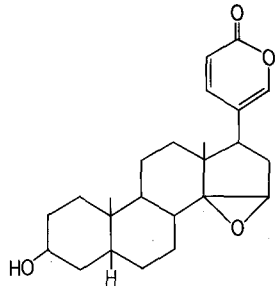
2a, R = H Amoorastatin
b, R = OH 12-Hydroxyamoorastatin
c, R = OAc



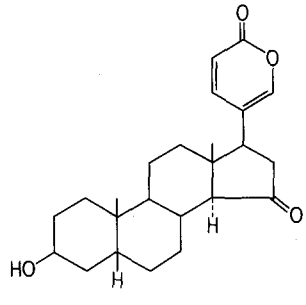
3 Amoorastatone



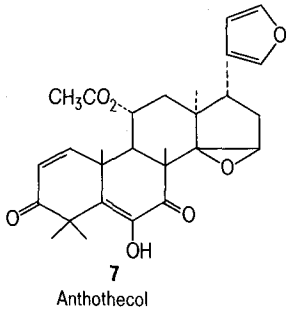
4 Sendanin



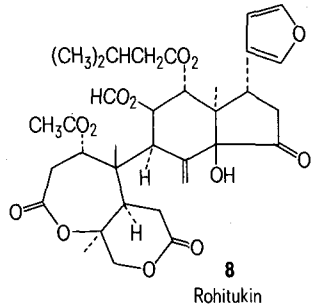
5 Resibufogenin



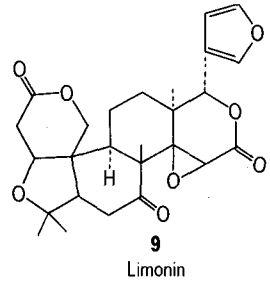
6 14α-Artebufogenin



7 Anthothecol



8 Rohitukin



9 Limonin

Table 2. ^{13}C-NMR spectrum of amoorastatone (3) measured at 22.6 MHz in deuteriochloroform-pyridine d_5 (~20%) recorded in ppm downfield from tetramethylsilane[6]

C-1	70.6	d	C-14	61.0	d
C-2	36.1	t	C-15	218.7	s
C-3	74.5	d	C-16	47.6**	t
C-4	40.4*	s	C-17	40.9	d
C-5	28.4	d	C-19	64.1	t
C-6	27.5	t	C-20	122.1	s
C-7	68.7	d	C-21	143.1	d
C-8	42.2*	s	C-22	110.5	d
C-9	51.3	d	C-23	140.2	d
C-10	44.5*	s	C-28	95.9	d
C-11	211.8	s	$COCH_3$	169.9	s
C-12	50.8**	t	$COCH_3$	23.5	q
C-13	45.7*	s	CCH_3	21.4	q
				20.1	q
				19.6	q

* and ** These values may be interchanged.

We are also pleased to report that in comparison cell growth (P388) inhibition studies sendanin (**4**)[7] was found quite (ED_{50} = 0.01 μg/ml) active, anthothecol (**7**)[14] significantly (ED_{50} = 1.2 μg/ml) active and both rohitukin (**8**)[15,16] and limonin (**9**)[17] were found to be inactive (ED_{50} > 100 μg/ml). Based on these observations, an intact steroid D-ring bearing a 14,15β-epoxide and the 17α-3′-furan system seem important structural requirements for inhibiting growth of the P388 lymphocytic leukemia cell line by limonoid-type triterpenes.

1 Part 63 of the series 'Antineoplastic Agents'. For the previous contribution refer to G.R. Pettit, T.S. Krupa and R.M. Reynolds, Int. J. Peptide Protein Res., in preparation. The present investigation was supported in part by Public Health Research Grant No. CA-16049-05 from the National Cancer Institute, the Fannie E. Rippel Foundation, Mrs Mary Dell Pritzlaff, the Spencer T. and Ann W. Olin Foundation, Mr John F. Schmidt, and the Phoenix Coca-Cola Bottling Company.

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16 We are pleased to thank Professors D.H.R. Barton and D.A.H. Taylor respectively for the specimens of limonin and rohitukin, and Dr C.L. Herald for general assistance.
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An Argentine ant aggregation factor

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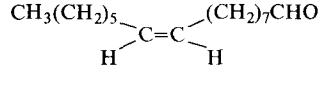
Summary. (Z)-9-Hexadecenal, a general aggregation factor of the Argentine ant, *Iridomyrmex humilis*, has been isolated and chemically characterized. It is implicated as a component of the trail-pheromone complex on evidence that it is a constituent of the ventral gland secretion, and that it both activates and attracts *I. humilis* workers.

Biological and chemical studies directed towards identifying the various aggregation factors produced by the Argentine ant, *Iridomyrmex humilis*, to activate and control trailing and other behavioural functions, have resulted in the isolation and characterisation of (Z)-9-hexadecenal (**1**).



(1)

The hexadecenal was obtained by total extraction of *I. humilis* workers with methylene chloride as previously described[3]. The extract, after removal of acidic constituents, was concentrated and then subjected to column chromatography on silica gel H. A lipid fraction, eluted with light petroleum/ether (90/10), had a characteristic fragrant odour and attracted *I. humilis* workers in multi-choice olfactometer tests. This fraction, predominantly glycerides, contained a number of trace constituents. Further column chromatography, followed by gas chromatography gave the hexadecenal (**1**), of linear retention index (LRI) 1770[4]. This trace constituent represents some 6 ppm of the body weight of the insect.

The mass spectrum of (**1**)[5] was consistent with the compound being a long chain unsaturated aldehyde of molecular formula $C_{16}H_{30}O$. It showed M^+ 238 (3%) and an M-18 ion m/e 220 (7%) indicative of the loss of the elements of water. The remainder of the spectrum was consistent with that of an alkene, with the base peak at m/e 55. Microhydrogenation of (**1**) (5 μg) gave only one product of increased retention time (LRI 1790). The mass spectrum of this product was indistinguishable from that of an authentic specimen of hexadecanal[6]. Micro-ozonolysis of (**1**)[7] (10 μg) yielded heptanal, identified by GC-MS. Compound (**1**) is 9-hexadecenal.