Structure of Ajugasterone C, a Phytoecdysone with an 11-Hydroxy-group[1]

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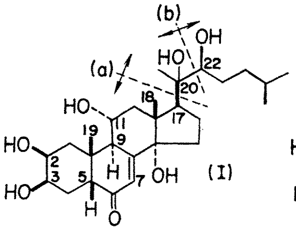
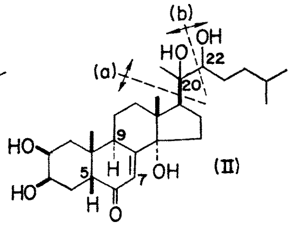
Summary The new phytoecdysone isolated from *Ajuga japonica* Miq. is shown to be 2β,3β,11α,14α,20,22-hexahydroxy-5β-cholest-7-en-6-one.

THE dried leaves (300g) of *Ajuga japonica* Miq. ("ohgikazura") afford ecdysterone (600 mg.), cyasterone (174 mg.),[2] and a new phytoecdysone, ajugasterone C (30 mg.)† to which we assign structure (I), *i.e.*, 11α-hydroxyponasterone A; its moulting activity as assayed by the *Chilo* dipping test[3] is 0·5—1μg./insect, comparable to other ecdysones. Ajugasterone C has also been obtained from the dried leaves of *Ajuga decumbens* Thunb. ("kiranso") (0·0015%).



(I)



(II)

Ajugasterone C (I), is noncrystalline, $C_{27}H_{44}O_7$ ($M^+ - 18$ at m/e 462); i.r. (KBr) 3400, 1655 cm.$^{-1}$; u.v. (MeOH) 243 nm. (ϵ 10,320); ajugasterone C 2,3,11,22-tetra-acetate is also noncrystalline, $C_{35}H_{52}O_{11}$ ($M^+ - 60$ at m/e 588).

Mass spectrometry provides a powerful tool in elucidating the structures of the closely related ecdysones. High-resolution mass spectrometry[4] has clarified several fissions of great diagnostic value (see Scheme), *e.g.*, fissions between C-17 and C-20 (a), C-20 and C-22 (b), C-23 and C-24 (only in C_{28}–C_{29} phytoecdysones having alkyl substituents at C-24); fission (b) is particularly useful because the two series of peaks differing in 18 mass units (H_2O) originating from the skeletal and side-chain fragments are quite intense.

As indicated in Table 1, the side-chain fragments arising from fission (a) and (b) were very similar to those of ponasterone A (II);[4,5] the identity of the side-chain of ajugasterone C (I) and ponasterone A (II), including the C-22 configuration, is established from the n.m.r. absorptions of the 20-methyl, 25-dimethyl and 22-H peaks (Tables 2, 3).

In contrast, the skeletal fragments arising from fissions (a) and (b) are 16 mass units higher in ajugasterone C than

TABLE 1

Mass spectroscopic fragmentations of (I) [*Ajugasterone C*] *and* (II) [*Ponasterone A*]

		Skeletal fragments			Side-chain fragments	
		(I)	(II)		(I)	(II)
(a)	17/20	335[a] (1)[b]	319[a] (1)[b]	(a) 17/20	145[a] (23)[b]	145[a] (13)[b]
		317 (2)	301 (7)		127 (6)	127 (5)
		299 (6)	283 (2)		109 (21)	109 (6)
		281 (4)				
(b)	20/22	379 (5)	363 (1)	(b) 20/22	101 (2)	101 (3)
		361 (16)	345 (6)		83 (25)	83 (20)
		343 (64)	327 (5)			
		325 (39)				

[a] *m/e*.
[b] Numerals in parentheses denote % intensities relative to base peak at *m/e* 43.

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† Ajugasterone A has been found to be identical with polypodine B: J. Jizba, V. Herout, and F. Šorm, *Tetrahedron Letters*, 1967, 5139. We thank Dr. Herout for a gift of polypodine B. For ajugasterone B, see ref. 1.

in ponasterone A, suggesting the presence of an extra oxygen function in the former compound. This is also clear from formation of a tetra-acetate in contrast to the formation of a triacetate from ponasterone A under normal conditions, and from the presence of an extra CH·OAc n.m.r. peak at 5·1—5·4 p.p.m., in the acetate spectrum (Table 3).

The u.v. and o.r.d. data are very similar to those of ponasterone A and other phytoecdysones possessing the familiar A/B-*cis*, 14α-hydroxy-7-en-6-one system; moreover, the 5β-H n.m.r. signal at 2·34 p.p.m. in the tetra-acetate, which should be very sensitive to the ring A conformation and substituents, was almost identical with that at 2·38 p.p.m. in (II)-triacetate (Table 3; the signals measured in perdeuterioacetone were also very similar). As in the case of ponasterone A, ajugasterone C also forms a diacetonide, and in conjunction with the similarity in the chemical shifts of the 5β-H n.m.r. signals of (I) and (II) acetates, the 2- and 3-hydroxy-groups in (I) are both β. Thus, excepting the presence of an extra hydroxy-group in the latter, the structures of the two ecdysones are identical.

The differences in the n.m.r. spectra of ajugasterone C and ponasterone A (and other ecdysones having identical skeletal structures) are seen in the chemical shifts of the angular methyl groups, especially C-19, and the signal-shape and chemical shift of the methine proton at C-9. The n.m.r. of ajugasterone C tetra-acetate (Table 3) shows that the 9-H at 3·39 p.p.m. is coupled to 7-H and 11-H with J 2·5 and 9 Hz., respectively (confirmed by decoupling); in contrast, the 9-H in ponasterone A triacetate is at 3·12 p.p.m. and is coupled to 7-H, 11α-H, and 11β-H. The extra hydroxy-group is thus at C-11, and in view of the J value of 9 Hz. (J_{aa}) and the ease of acetylation, it is equatorial, *i.e.*, 11α-OH (the spatial dispositions of the 2-, 3-, and 11-hydroxy-groups are corroborated[6] by the dibenzoate chirality rule). The shift in the n.m.r. position of the 10-Me group, as compared with other ecdysones, is also due to this extra hydroxy-group. Presence of the 11-hydroxy-group is supported by the behaviour of the tetra-acetate upon heating in Al_2O_3–benzene to give a product absorbing in the u.v. at 298 nm.; the calculated value for the 7,9(11)-dien-6-one chromophore is 303 nm. Ajugasterone C is the first phytoecdysone having an extra substituent on ring C.

TABLE 2. *Methyl chemical shifts of free compounds* (δ, *p.p.m.*)

(deuteriopyridine solution; numerals in parentheses are J values in c./sec.)

	C-18	C-19	C-21	C-26/27
Ajugasterone C (I)	1·21	1·27	1·51	0·82 (d, 5)
Ponasterone A (II)	1·16	1·06	1·55	0·82 (d, 6)

TABLE 3. *Chemical shifts of the acetates* (δ, *p.p.m.*)

(deuteriochloroform solution; numerals in parentheses are J values in c./sec.)

Methyl protons

	C-18	C-19	C-21	C-26/27
(I)-2,3,11,22-Tetra-acetate	0·89	1·10	1·23	0·88 (d, 6)
(II)-2,3,22-Triacetate	0·85	1·02	1·24	0·88 (d, 6)

Carbinyl and olefinic protons

	C-2	C-3	C-11	C-5	C-7	C-9	C-22
(I)-2,3,11,22-Tetra-acetate	5·1	—	5·4[a]	2·34	5·88	3·39	4·79
				(d, d 7·0, 10·0)	(d 2·5)	(d, d 2·5, 9·0)	(d,d 3·5, 9·0)
(II)-2,3,22-Triacetate	5·05	5·32	—	2·38	5·86	3·12	4·82
	(d, d, d 11·5, 4·5, 3·5)	(d, d, d 4·0, 3·8, 3·5)		(d, d 6·0, 11·0)	(d 2·5)	(d, d, d 2·5, 8·0, 10·0)	(d, d 3·5, 9·0)

[a] J Values could not be measured because of overlap of three signals.

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[1] Previous paper: S. Imai, S. Fujioka, E. Murata, K. Otsuka, and K. Nakanishi, *Chem. Comm.*, 1969, 82.

[2] T. Takemoto, Y. Hikino, K. Nomoto, and H. Hikino, *Tetrahedron Letters*, 1967, 3191; S. Imai, T. Toyosato, M. Sakai, Y. Sato, S. Fujioka, E. Murata, and M. Goto, *Chem. and Pharm. Bull.* (*Japan*), 1969, 17, 340.

[3] Y. Sato, M. Sakai, S. Imai, and S. Fujioka, *Appl. Ent. Zool.*, 1968, 3, 49.

[4] M. Koreeda, K. Nakanishi, K. Morita, S. Imai, T. Tsuchiya, and N. Wasada, 4th Mass Spectrometry Symposium (Japan), Nagoya, November, 1968, Abstracts, p. 5. (Paper to be published.)

[5] K. Nakanishi, M. Koreeda, S. Sasaki, M. L. Chang, and H. Y. Hsu, *Chem. Comm.*, 1966, 915.

[6] M. Koreeda, N. Harada, and K. Nakanishi, following communication.