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29-NORSENGOSTERONE AND 29-NORCYASTERONE, NEW C-28 PHYTOECDYSTEROIDS FROM AJUGA REPTANS (LABIATAE)[1]

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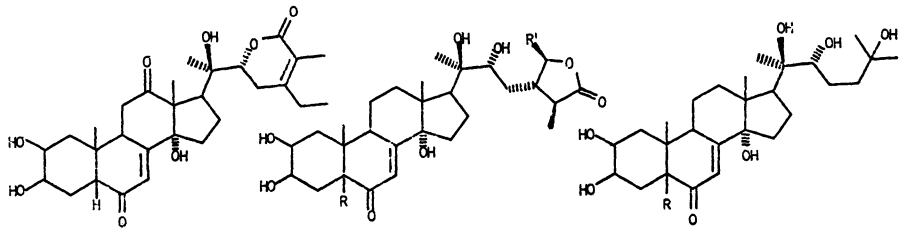
Ajugalactone (1), cyasterone (3), polypodine B (5), β-ecdysone (6) and two new C-28 phytoecdysteroids, 29-norsengosterone (2) and 29-norcyasterone (4), have been isolated from Ajuga reptans and characterized by spectral means and X-ray diffraction analysis of 2,3,20,22-diacetonide of 4.

Following our studies on allelochemicals of Ajuga plants[2], we report herein the isolation of phytoecdysteroids from Ajuga reptans (Labiatae).

Fractionation of an ethanol extract of 1.180 Kg of the whole dry plant, collected at Montseny (Barcelona), by reversed phase chromatography, according to a well established procedure[3], allowed us to concentrate the insect moulting activity in the 33 and 70% methanol fractions. Repeated chromatography of these combined fractions on silica gel using a 5 : 1 $CHCl_3$: MeOH elution mixture afforded the following pure phytoecdysteroids, in order of increasing polarity (% yield based on dry plant): ajugalactone (1) (0.0068%), 29-norsengosterone (2) (0.0066%), cyasterone (3) (0.0026%), 29-norcyasterone (4) (0.012%), polypodine B (5) (0.0039%) and β-ecdysone (6)(0.0066%).[4] The elucidation of the structures of other two minor phytoecdysteroids is now in progress. When the above fractionation was carried out according to S. Imai et al.[5], we obtained a remarkable increase in the isolation yield of the less polar phytoecdysteroids, 1 (0.0094%) and 2 (0.011%).

Figure 1



Furthermore, peaks at m/z 187,169 and 143 in the MS of 2 and 4, shifted 14 m.u. to lower values when compared with the MS of cyasterone (3)[6], were consistent with the replacement of one methyl group by one hydrogen in the corresponding side chains, as confirmed by comparison of ^{1}HNMR methyl absorptions in the spectra of 2, 3, 4 and sengosterone (7)[10] (Table 2).

Table 2.- ^{1}HNMR data of methyl absorptions

Compound	C-19	C-29	C-27	C-18	C-21
4	1.04 (s)		1.18 (d, J = 7 Hz)	1.20 (s)	1.55 (s)
3	1.06 (s)	1.33 (d, J = 7 Hz)	1.35 (d, J = 7 Hz)	1.19 (s)	1.58 (s)
2	1.13 (s)		1.18 (d, J = 7 Hz)	1.13 (s)	1.53 (s)
7	1.13 (s)	1.34 (d, J = 7 Hz)	1.36 (d, J = 7 Hz)	1.21 (s)	1.56 (s)

Finally, the structures of 29-norsengosterone and 29-norcyasterone could be respectively inferred for compounds 2 and 4 from the absence of peak at δ 19.3 assigned to C-29 in the corresponding ^{13}CNMR spectra (Table 1).

On the other hand, the assignment of relative configurations at C-24 and C-25 in compounds 2 and 4 was substantiated by the identity of chemical shifts and coupling constants of methine H-22, methylene H-28 and methyl H-27 in the ^{1}HNMR spectra of 2,3,22-triacetates of 2 and 4 with those of the 3,22-diacetate of 25-epi-perulactone (9) formed from the recently described withanolide perulactone (8)[11], by base isomerization at C-25 and acetylation (Figure 2).

The presence of 3, as well as the determination of 24-S configuration for clerosterol (10) and 22,23-didehydroclerosterol (11), the main sterols isolated from Ajuga reptans[12], verified the assignment of configurations 20R, 22R, 24S, 25S for compounds 2 and 4.

The structure of 4 has been further confirmed by X-ray diffraction analysis of its 2,3,20 22-diacetonide (Figure 3)[13]. Crystals are orthorhombic $P2_12_12_1$, a = 7.42 (1), b = 11,25 (3), c = = 40.39 (16)Å, V = 3374 (18)Å^3, Z = 4. The structure was determined with the computer program MULTAN-80 system[14] and refined to R = 0.079 for 1124 observed reflections, using the SHELX program system[15].

It is noteworthy that polypodine B (5) had not been previously reported in Ajuga plants and that 2 and 4 are the first C-28 phytoecdysteroids found with a lactone moiety in the side chain.

Figure 2

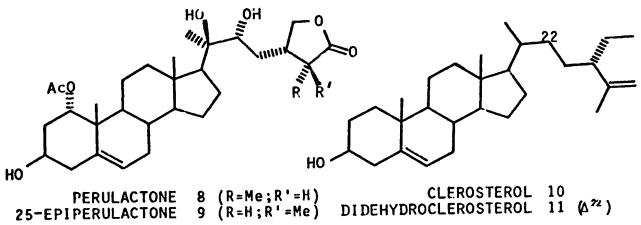
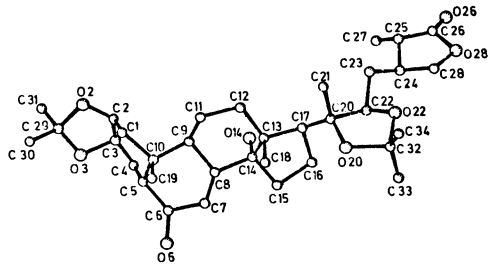


Figure 3.- PLUTO prespective drawing of 2,3,20,22-diacetonide of 4.



Structures 3, 5 and 6 were ascertained by direct comparison of the corresponding chromatographic and spectral features of our compounds with those of authentic samples[6-8]. Likewise, the identification of 1, [m.p. 255-60° (d, MeOH), $[\alpha]_D$=+86.5° (c 5.71, MeOH), M^+ 516; 2,3-diacetate, m.p. 237-43° (d, MeOH), $[\alpha]_D$=+83.9° (c 5.62, MeOH), M^+ 600] was substantiated by agreement of our spectral data (UV, IR, MS and ^{1}HNMR) with those described for ajugalactone[9], as well as by complete analysis of the ^{13}CNMR spectrum determined with our sample (Table 1).

The structures of the heretofore unreported 29-norsengosterone (2) and 29-norcyasterone (4) were established by spectral means and confirmed in the case of 2,3,20,22-diacetonide of 4 by X-ray diffraction analysis.

Compound 4, $C_{28}H_{42}O_8$ [M^+-H_2O 488, m.p. 152-5° (MeOH), $[\alpha]_D$=+32.4° (c 6.25, MeOH), CD $[\theta]_{340}$= =+49 x 10^2 (c 0.36, dioxane); 2,3,20,22-diacetonide, M^+ 586, m.p. 264-6°(d, MeOH), $[\alpha]_D$=+18.2 (c 2.73, MeOH); 2,3,22-triacetate, M^+-$2H_2O$ 596, m.p. 153-6°C (MeOH), $[\alpha]_D$=+44.5°(c 3.12, MeOH)] contained a ring identical to that found in phytoecdysteroids 3 and 6 as shown by the following spectral data: m/z 363, 345, 327 and 309, arising from a C_{20}-C_{22} cleavage[6]; λ_{max} (MeOH) 241 nm (ε9800) shifted to 290 nm after treatment with methanolic hydrochloric acid; ν_{max} (KBr) 1650 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ6.25 (d, J = 2 Hz) and ^{13}CNMR δ203.9 (s), 166.2 (s), and 121.9 (d) (Table 1), in accord with the occurrence of a 14α-hydroxy-7-en-6-one system.

Compound 2, $C_{38}H_{42}O_9$ [M^+-H_2O 504, amorphous solid, $[\alpha]_D$=+51.0° (c 4.7, MeOH), CD $[\theta]_{330}$= =+65 x 10^2 (c 0.35, dioxane); 2,3,20,22-diacetonide, M^+ 602, m.p. 149-52° (MeOH), $[\alpha]_D$=+38.8° (c 2.7, MeOH); 2,3,22-triacetate, M^+ -$2H_2O$ 612, amorphous solid, $[\alpha]_D$=+23.3°(c 3.7, MeOH)] exhibited a ring system identical to that found in polypodine B (5) as inferred from the following spectral data: m/z 361, 343 and 325, shifted 16 m.u. when compared to the above sequence, pointing to the presence of an extra hydroxyl group; λ_{max} (MeOH) 238 nm (ε 9000) shifted to 290 nm after treatment with methanolic hydrochloric acid; ν_{max} (KBr) 1670 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ6.25 (d, J = 2 Hz) and ^{13}CNMR δ201.0 (s), 166.7 (s) and 120.0 (d), in accord with the occurrence of a 14 α-hydroxy-7-en--6-one system, and 79.8 (s), in agreement with the presence of a 5β-hydroxyl group (Table 1).

In addition, the existence of a γ-lactone functionality in the side chain of compounds 2 and 4 was also evident from the corresponding spectral absorptions: ν_{max} (KBr) 1755 and 1750 cm^{-1}, ^{13}CNMR δ180.1 (s) and 179.8 (s). Likewise, the occurrence of a 20,22-diol moiety was inferred from the intense peak at m/z 227 in the MS of both diacetonides.

Table 1.- ^{13}CNMR data of compounds 1-6 (*,','' : assignments can be interchanged).

C	1	2	3	4	5	6	C	1	2	3	4	5	6
1	37.9t	34.7t	37.9	38.0t	34.7	38.2t	16	21.3t	21.3t'	21.0'	21.7'	21.3'	21.7 '
2	67.9d	67.9d	68.0	68.2d	67.9	68.5d	17	44.1d	50.0d	50.0	50.3d	49.9	50.5d
3	68.2d	69.8d	68.0*	68.2d *	69.7	68.3d*	18	17.7q	17.9q	17.8	18.0q	17.8	18.2q
4	32.3t *	35.9t	32.0*	32.6	35.9	32.3	19	24.0q	17.1q	24.4	24.6q	17.1	24.7q
5	51.5d	79.8s	51.3	51.5d	79.8	51.7d	20	75.3s	76.7s	76.7	76.9s	75.7	77.2s
6	202.7s	201.0s	203.7	203.9s	200.9	203.9s	21	22.5q	21.3q	21.1'	21.4'	21.6'	21.4 '
7	124.3d	120.0d	122.2	121.9d	119.8	121.9d	22	83.4d '	76.0d	73.9	76.2d	77.5	77.8d
8	162.4s	166.7s	166.1	166.2s	166.7	166.6s	23	27.4t	34.7t	34.4	34.8t	27.4	27.7t
9	37.1d	38.2d	34.8	34.6d	38.2	34.7d	24	121.4s	40.7d	48.1	40.5d	42.5	42.8t
10	40.1s	44.8s	38.6	38.8s	44.7	39.0s	25	154.9s	43.3d	42.2	43.5d	69.5	70.0s
11	37.1t	22.0t'	21.3	21.6 '	22.0 '	22.0 '	26	167.3s	179.8s	179.3	180.1s	30.0''	30.4q''
12	210.1s	31.7t*	31.8*	31.9 *	31.5 *	32.0 *	27	11.8q''	14.5q	15.8	14.6q	29.9''	30.2q''
13	61.9s	48.2s	48.7	48.3s	48.0	48.4s	28	20.4t '	72.9t	79.8	73.1t	-	-
14	89.5s	84.0s	84.1*	84.3s	83.9	84.5s	29	12.5q''	-	19.3	-	-	-
15	32.2t*	32.1t*	32.4*	32.1 *	32.0 *	32.7 *							

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References

1.- Preliminary results of this study have been presented at the IUPAC 12th International Symposium on the Chemistry of Natural Products (Puerto de la Cruz, Tenerife, Spain, 1980) p.149 (B48).

2.- F. Camps, J. Coll and A. Cortel, Chem. Lett. 1093 (1981)

3.- D.A. Schooley, G. Weiss and K. Nakanishi, Steroids 19, 377 (1972)

4.- Both methanol fractions contained different amounts of 2-6, while 1 was only found in the 70% fraction. After this work was completed, we have been informed by Prof. I. Kubo about the isolation of some phytoecdysteroids from Ajuga reptans by straight application of countercurrent droplet-chromatography.

5.- S. Imai, S. Fujioka, K. Nakanishi, M. Koreeda and T. Kurokawa, Steroids, 10, 557 (1967)

6.- H. Hikino, Y. Hikino, K. Nomoto and T. Takemoto, Tetrahedron, 24, 4895 (1968)

7.- H. Rimpler, Tetrahedron Letters 329 (1969)

8.- H. Hoffmeister and H.F. Grützmacher, Tetrahedron Letters 4017 (1966)

9.- K. Nakanishi, M. Koreeda and M. Goto, J.Am.Chem.Soc., 92, 7512 (1970)

10.- H. Hikino, K. Nomoto and T. Takemoto, Tetrahedron, 26, 887 (1970)

11.- H.E. Gottlieb, I. Kirson, E. Glotter, A.B. Ray, M. Sahat and A. Ali, J.C.S. Perkin I, 2700 (1980)

12.- F. Camps, J. Coll and A. Cortel, to be submitted for publication.

13.- C. Miravitlles, X. Solans, G. Germain and J.P. Declercq, Acta Crystallogr. Commun. to be submitted.

14.- P. Main, S.J. Fioke, S.E. Hull, L. Lessinger, M.M. Woolfson, G. Germain and J.P. Declercq. Multan 80. A system of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data. Universities of York (England) and Louvain-La-Neuve (Belgium) (1980)

15.- G.M. Scheldrick, SHELX76 Program for Crystal Structure determination University of Cambridge (England) (1976)

16.- S. Motherwell and V. Clegg. PLUTO, University of Cambridge (England) (1978)

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