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TWO NEW CLERODANE DITERPENOIDS FROM AJUGA REPTANS (LABIATAE)

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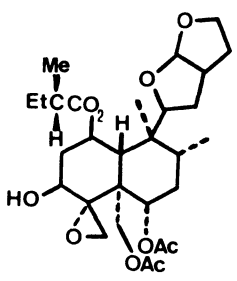
Ajugareptansone A and B, 2 and 3, two new neo-clerodane diterpenoids have been isolated from Ajuga reptans, and their structures have been elucidated by spectral methods and X-ray diffraction analysis. Under conditions used for isolation and purification of these compounds, formation of 3 from 2 has been observed.

A renewed interest in the study of clerodane diterpenoids has been originated by the diverse biological activities exhibited by these compounds. In this context, some previous absolute configuration assignments have been revised and a modified nomenclature has been proposed[1-3].

Recently, we have elucidated the structure of ajugareptansin 1, a new neo-clerodane diterpenoid isolated from Ajuga reptans, by chemical and spectral means[4]. The absolute configuration of this compound has been confirmed by X-ray analysis of the corresponding p-bromobenzoate[5].

In the present communication we report the isolation from the same plant of two new diterpenoids with neo-clerodane structure, closely related to ajugarins[6].



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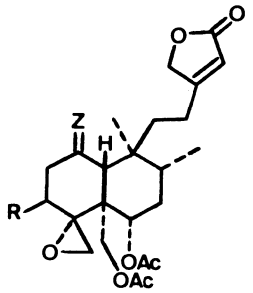
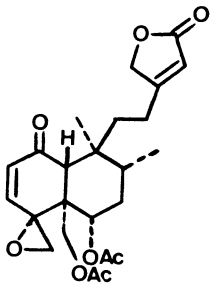


2: R = $EtMeCHCO_2$-; Z = O

4: R = H; Z = H_2



3

The residue from a diethyl ether extract of air dried whole plant was chromatographed on alumina (Brockmann grade V). Waxes and carotenes were eluted

by hexane/diethyl ether grádient solvent system (1:0 to 1:1). Further elution with hexane/ethyl acetate gradient solvent system (10:1 to 0:1) afforded crude diterpenoid fractions. The joined fractions were rechromatographed on alumina (Brockmann grade II) eluting with a 1:1 hexane/ethyl acetate mixture to yield ajugareptansone A, 2 (0.0008%), as colorless prisms, m.p. 177-180º (ethyl acetate/diethyl ether). Further elution with 2:3 and 1:2 hexane/ethyl acetate mixtures gave, after evaporation and dilution of the residue with ethyl acetate, ajugareptansone B, 3 (0.0006%) as colorless prisms, m.p. 255-263º(d). Solvent removal of the mother liquor and crystallization from diethyl ether afforded ajugareptansin (0.05%) aa colorless needles, m.p. 148-150º (acetate, m.p. 141-143º (diethyl ether); benzoate, m.p. 162-165º, 1a (ethanol)). It is worth of note that the present modified isolation procedure doubled the yield of ajugareptansin 1 previously obtained.

MS and microanalytical data of ajugareptansone A indicated a molecular formula of $C_{29}H_{40}O_{10}$ (M^+, 548. Calcd. C, 63.5%; H, 7.6% and Found C, 63.3%; H, 7.5%. $[\alpha]_D$=-6º ($CHCl_3$, c 1.98). The UV spectrum, λ_{max}(MeOH) 220 nm (ε 5500), pointed to the presence of an α,β-unsaturated carbonyl group, further confirmed by the strong 1785 and 1645 cm^{-1} IR absorptions, characteristic of a γ-butenolide moiety. Other broad strong bands at 1745 and 1240 cm^{-1} and a shoulder at 1725 cm^{-1} suggested the occurrence of acetate and other carbonyl groups in the molecule.

The ^{1}H-NMR spectrum (250 MHz, $CDCl_3$) showed absorptions at δ 4.70 (2H, s) and 5.82 (1H, s, $W_{1/2}$=6 Hz) verifying the presence of a β-substituted γ-butenolide, as reported in the structure of 4. Likewise, bands at δ 2.02 (3H, s), 2.06 (3H, s), 4.31 and 4.73 (2H, AB system, J=13 Hz) and 4.94 (1H, dd, J=10 and 6 Hz) confirmed the occurrence of one primary and one secondary acetate groups, whereas the presence of a terminal epoxide could be ascertained from the AB system appearing at δ 3.02 and 3.22 (2H, J=4 Hz). The methyl absorptions at δ 0.87 (3H, t, J=7 Hz) and the signal at δ 5.60 (1H, dd, J=10 and 6 Hz), as well as the m/z 446 peak (M-102) in the MS, pointed to the presence of an equatorial 2-methylbutanoic acid ester, located at the C-3 position, as inferred from the 5.60 signal.

As shown in the Table, the ^{13}C-NMR spectrum revealed the presence of a ketone absorption signal at δ 202.2, in agreement the 1725 cm^{-1} shoulder in the IR spectrum. The single proton absorption at δ 2.37 in the ^{1}H-NMR spectrum restricted the position of this keto group to position C-1. Two other methyl groups appearing at δ 0.92 (d, J=7 Hz) and 1.04 (s) were assigned to C-20 and C-19 respectively.

From comparison of the above data and the ^{13}C-NMR absorptions (Table) with those of 1 and 4, the structure 2 was inferred for ajugareptansone A. X-ray diffraction analysis confirmed the structure, with a neo-clerodane absolute configura-

TABLE

C	1	1a	4	2	C	1	1a	4	2
1	69.5 d	69.3	21.9	202.0	11	83.6 d	84.2	32.6[b]	35.0
2	37.9 t	35.7	21.0	46.0	12	33.9 t[a]	33.9	34.7	22.8
3	63.7 d	66.5	25.0	64.8	13	41.0 d	40.8	173.5	173.5
4	66.5 s	64.3	65.0	65.6	14	34.1 t[a]	33.9	115.7	115.6
5	44.7 s	44.8	45.2	45.1	15	67.7 t	68.0	170.7	169.5
6	71.3 d	70.9	72.2	72.7	16	108.2 d	108.2	72.9	71.0
7	32.6 t	32.8	32.9[b]	33.8	17	43.5 t	44.4	48.5	46.9
8	32.7 d	33.6	34.7	32.5	18	61.5 t	62.1	61.8	64.2
9	41.5 s	41.4	38.4	38.1	19	18.6 q	17.6	17.3	16.3
10	51.7 d	52.0	48.2	56.6	20	14.2 q	15.1	15.3	14.4
CH_3CO_2	21.2 q	21.1		21.0	CH_3CHCO_2	15.8 q	15.4		15.8
CH_3CO_2	21.2 q	21.1		20.4	$CH_3CH_2CHCO_2$	11.4 q	11.3		11.3
CH_3CO_2	170.0 s	170.2		169.8	$CH_3CH_2CHCO_2$	26.9 t	26.6		26.6
CH_3CO_2	169.4 s	169.8		169.5	$EtMeCHCO_2$	42.0 d	41.9		41.0
					$EtMeCHCO_2$	175.2 s	174.8		174.7

Spectra registered in $CDCl_3$, at 23.5 MHz (1) and 63.0 MHz (1a and 2) and from ref. 6 (4). Values in δ scale, relative to TMS. Assignments may be interchanged (a,b)

tion. Crystals of this compound are orthorhombic, $P2_12_12_1$, a=16.225 (3), b=11.007 (3), c=16.401 (3) Å, and Z=4. The structure was solved with the MULTAN method and refined to an R of 0.057 for 3497 reflections whereas the absolute configuration was determined by the Bijvoet-differences method. In the trans decalin system both rings adopt chair conformations[7].

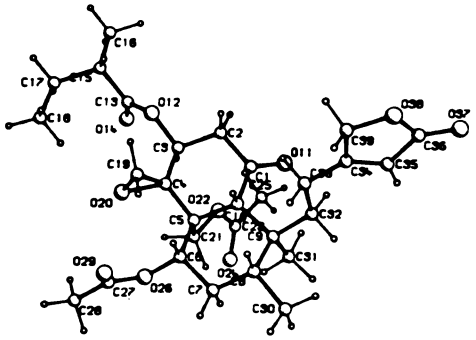
Ajugareptansone B has a molecular formula $C_{24}H_{30}O_8$ (M^+, 446. Calcd. C, 64.4%; H, 6.7%. Found C, 64.7%; H, 6.9%) and the following spectral features: UV λ_{max}(MeOH) 215 nm (ε 12000) and 325 nm (ε 1600); IR (KBr) 1785, 1750, 1720, 1680, 1645 and 1240 cm^{-1}; ^{1}H-NMR (250 MHz, $CDCl_3$) δ0.90 (3H, d, J=6 Hz), 1.09 (3H, s), 1.98 (3H, s) 2.04 (3H, s), 2.60 (1H, s), 2.77 and 3.51 (2H, AB system, J=4 Hz), 4.48 and 4.70 (2H, AB system, J=13 Hz), 4.73 (2H, s), 4.96 (1H, dd, J=10 and 6 Hz), 5.96 (1H, s, $W_{1/2}$=6 Hz), and 6.11 (2H, s).

Comparison of this NMR spectrum with that of 2 revealed the presence of the same functionality as above, except for the absence of the 2-methylbutanoate group. On the other hand, the presence of a vinylic proton absorption at δ 6.11 (2H) and the identity of MS fragmentation pattern between 2 and 3 substantiated the proposed structure for the latter. It is noteworthy that 3 exhibited some unexpected spectral features, namely, the strong 325 nm UV absorption, the 1720 and 1680 cm^{-1} IR bands and the magnetic equivalence of the vinylic protons at δ 6.1. These features were attributed to the presence of the epoxide ring which could exert an electronic effect and also induce a deviation from coplanarity (ca. 15º) on the -C=C-C=O moiety[8].

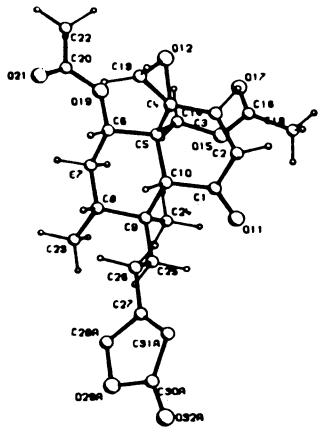
The structure of 3 has been confirmed by X-ray diffraction analysis. Crystals are orthorhombic $P2_12_12_1$, a=10.681 (8), b=9.758 (7), c=21.320 (13) Å, Z=4.

The structure was determined with Patterson-Search method, using as reference the structure of ajugareptansone A, and refined to an R=0.067 for 865 observed reflections. Rings of the trans decalin system adopt envelope and chair conformations[8].

The close structural relationship between 2 and 3 prompted us to check the possible formation of 3 during the isolation and purification procedures. This possibility was confirmed by admixing a 1:2 hexane/ethyl acetate solution of 2 (0.18 mg/mL) with alumina (Brockmann grade I)(60 mg), and leaving the mixture standing at room temperature. HPLC analysis (ODS-HC Silx-I column, p=1700 psi, 225 nm detection, elution with a mixture of methanol/water 51% at 1 mL/min) revealed the presence of 25% and 55% of 3 after 24 and 48 hours. However, the presence of 3 in the plant cannot be conclusively excluded after a direct analysis of the crude extract.



ORTEP perspective drawing of **2**



ORTEP perspective drawing of **3**

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