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AJUGASTERONE C AND 5-DEOXYKALADASTERONE, AN ECDYSTEROID ARTIFACT, FROM *LEUZEA CARTHAMOIDES*

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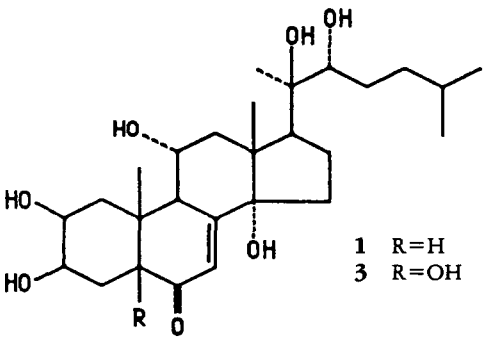
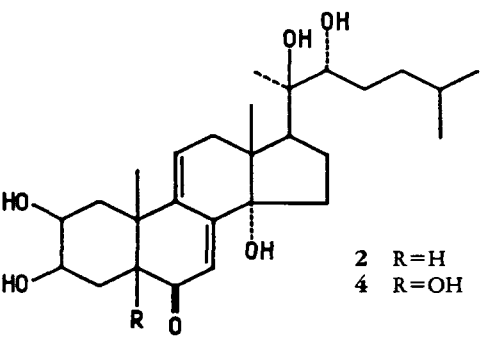
Ecdysteroids are polyhydroxylated steroids containing, with very few exceptions, as many as 4–7 hydroxyl groups on the sterol nucleus (1). Their separation and isolation from the complex biological matrix usually require a multistep purification process using various chromatographic sorbents/supports, the most commonly used being Si gel and alumina. The present paper describes the isolation of ajugasterone C [**1**], an 11α-OH-derivative, from the roots of *Leuzea carthamoides* DC. (Asteraceae) and reports on the observed conversion of **1** into the dehydration product **2**, which was also isolated from the same extract using alumina for the separation of ecdysteroids.

Ajugasterone C [**1**] was identified through its spectral data and direct comparison with an authentic sample (3). This seems to be the first report of ajugasterone C from the Asteraceae family.

Compound **2** was identified on the basis of its spectral characteristics as 25-deoxy-9(11)-dehydro-20-hydroxyecdysone. Its unusual uv spectrum with a maximum at 298 nm fits well with data reported earlier for kaladasterone [**4**] (4),



1 R=H
3 R=OH



2 R=H
4 R=OH

and the $\Delta^{9(11)}$ structure was established by its ^{1}H-nmr spectrum (Table 1). The unusual dienone character of the tetracyclic nucleus of **2** raised some doubt as to its authentic nature. The corresponding 5-hydroxy derivative (kaladasterone [**4**]) was also found together with muristerone A [**3**] in kaladana seeds [seeds of various *Ipomoea* species (Convolvulaceae)] (4), and the authors concluded that both compounds were present in the plant as native compounds.

of the freshly made extracts (fresh or dry plant) by tlc. Besides this study, it was previously reported that heating of ajugasterone C in C_6H_6 with alumina resulted in the formation of a product with a uv absorption maximum at 298 nm that was not fully characterized but was thought to bear a 7,9(11)-dien-6-one chromophore (5). Both ajugasterone C [**1**] and compound **2** (named as dacryhainansterone) have previously been reported from *Dacrydium pierrei* Hickel

TABLE 1. The ^{1}H-nmr Spectra of Compounds **1** and **2** in CD_3OD.[a]

Proton	Compound	
	1	**2**
1-H_a	1.39	1.7
1-H_e	2.59 (dd, 13,4.2)	2.1
2-H_a	4.01 (m, W½ = 22)	3.72
3-H_e	3.96 (m, W½ = 8)	3.84
4-H_a	1.72	1.6
4-H_e	1.89	1.78
5-H	2.35 (dd, 12,5)	2.40
7-H	5.80 (d, 2.5)	5.75 (s, br, W½ = 4)
9-H_a	3.15 (dd, 8.8, 2.8)	—
11-H_a	4.07 (m, W½ = 28)	6.3 (ddd, 6.5, 2,2)
12-H_a	2.22 (ddd, 13,13,5)	2.72 (d, br, 18)
12-H_e	2.16	2.42
15-H_a	1.95	
15-H_b	1.58	
16-H_a	1.98	2.05
16-H_b	1.74	1.75
17-H	2.41 (m)	2.45
22-H_b	3.32 (dd, 11,2)	3.33 (dd, 11,2)
23-H_a	1.22	1.35
23-H_b		1.55
25-H	1.55	1.55
18-Me	0.88 (s)	0.89 (s)
19-Me	1.06 (s)	1.10 (s)
21-Me	1.20 (s)	1.18 (s)
26-Me	0.92 (d, 6.4)	0.91 (d, 6.5)
27-Me	0.93 (d, 6.4)	0.94 (d, 6.5)

[a]Multiplicity of signals: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad signal. W½: width at half-height in Hertz; δ in ppm.

In the present study, however, we consider that it is highly probable that the presence of **2** is an artifact formed during the multistep purification process. This assumption is based on the cooccurrence of **2** and **1** in the same plant, the easy conversion of **1** into **2**, and the fact that **2** could not be detected in any

(Podocarpaceae) (6). It is suggested that the name 5-deoxykaladasterone be retained for compound **2** indicating its genetic relationship to kaladasterone [**4**] as well as to ajugasterone C [**1**]. As indicated earlier, the authentic nature of $\Delta^{9(11)}$-ecdysteroids has repeatedly been questioned (2). Our present studies seem

to confirm that in each case when 11α-OH derivatives have been found together with the corresponding 11-dehydro derivatives, the latter were probably formed during the purification process and should, thus, be considered as artifacts.

EXPERIMENTAL

ISOLATION OF **1** AND **2**.—The extraction and the isolation processes of the main ecdysteroids were described in detail in a previous paper (2). Briefly, the ecdysteroid mixture was purified by solvent extraction and by precipitation, then filtered through an Al_2O_3 column. The ecdysteroids were eluted as a mixture by CH_2Cl_2-EtOH (9:1); 500-mg portions of the purified mixture underwent dccc separation using a $CHCl_3$-C_6H_6-MeOH-H_2O (15:15:23:7) system in the descending mode. One pure compound **2** was obtained as white needles from MeOH (34 mg): mp 242–245°; uv λ max (EtOH) 298 (ε 14,200), 235 (ε 6400) nm; ir ν max (KBr) 3200–3550, 2940, 2860, 1620, 1600, 1450, 1445, 1365, 1355, 1290, 1245, 1125, 1105, 1095, 1040, 1020 cm^{-1}; cims *m/z* $[M]^+$ 462; ^1H nmr see Table 1.

Further amounts of **2** together with ajugasterone C [**1**] were obtained from the same ecdysteroid mixture by separating the components on a Si gel column [CH_2Cl_2-EtOH (9:1) as eluent] and then on alumina preparative plates in an EtOAc-MeOH-NH_3 (85:10:5) system. The bands of **1** and **2** were eluted with MeOH and crystallized from the same solvent; 5 mg of **1** and 9.5 mg of **2** were obtained as a white, amorphous powder. Ajugasterone C [**1**] was identified by comparison with an authentic sample using hplc, ms, and ^1H nmr (see Table 1).

CONVERSION OF AJUGASTERONE C [**1**] INTO 5-DEOXYKALADASTERONE [**2**].—Compound **1** (1 mg) was dissolved in 2 ml of MeOH, and the solution was applied to 10 preparative Al_2O_3 F_{254} plates in band form. The plates were developed in EtOAc-MeOH-NH_3 (85:10:5), the ajugasterone C band (detected by its uv absorption at 254 nm) was scraped off, and the sorbent was mixed with 10 ml MeOH for 12 h. After filtering and evaporating the solvent, the residue was analyzed by tlc and hplc [Zorbax-SIL® 250 mm long, 9.2 mm i.d., solvent CH_2Cl_2-iPrOH-H_2O (125:30:2), flow rate 4 ml/min, retention volumes for **1** and **2** 72 ml and 34.4 ml, respectively]. Compounds **1** and **2** were present in an approximate ratio of 1:2, together with several minor uv-absorbing peaks that were not further investigated.

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