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A PHYTOECDYSTEROID, TAXISTERONE, FROM *TAXUS CUSPIDATA*

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Key Word Index—*Taxus cuspidata*; Taxaceae; phytoecdysteroid; taxisterone; 22-deoxyecdysterone.

Abstract—A new phytoecdysteroid, taxisterone, was isolated from *Taxus cuspidata* and the structure was deduced to be 22-deoxyecdysterone by spectral data.

INTRODUCTION

Air-dried leaves and stems of *Taxus cuspidata* have been used in folk medicine for therapy of diabetes. Ecdysterone (**3**) and ponasterone A have been isolated previously from this plant [1]. We have now isolated a new phytoecdysteroid and determined its structure from spectral data.

RESULTS AND DISCUSSION

The methanolic extract was prepared from the dried leaves and stems and separated as described in the Experimental to afford a new phytoecdysteroid, designated taxisterone (**1**). Taxisterone (**1**), a white powder (mp 120–125°), showed IR spectrum absorptions due to the hydroxyl (3400 cm^{-1}) and the α, β-unsaturated carbonyl (1650 cm^{-1}) groups. The ^1H NMR spectrum of the acetate (**2**) of **1** ($CDCl_3$) exhibited an analogous pattern to those [2] of phytoecdysteroids. In its spectrum, the respective signals could be assigned as shown in Table 1 by comparison with that [2] of ecdysterone 2, 3, 22-triacetate (**4**).

The signals due to the respective acetoxyls and carbinol methines at C-2 and C-3 were observed but no acetyl carbinol methine ascribable to C-22 was observed and a hydroxyl was assumed to be absent from C-22 in compound **1**. Moreover, the ^{13}C NMR spectrum of **2** ($CDCl_3$) showed five signals at δ 67.0(*d*), 68.7(*d*), 71.0(*s*), 75.0(*s*) and 84.8(*s*) due to the carbons attached to the oxygen function groups (Table 2).

In comparison with the ^{13}C NMR spectrum of **4**, it is evident that no carbon signal attributable to C-22 bearing a hydroxyl was detected. Therefore, **1** is represented as 22-deoxyecdysterone. As for ecdysones lacking the hydroxyl at C-22, 2, 22-dideoxy-20-hydroxyecdysone [3] has been recently obtained from the ovaries of the silkworm *Bombyx mori*. However, taxisterone (**1**) is the first example from the plant kingdom.

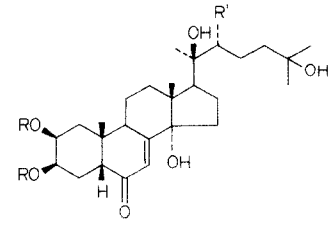
Table 1. ^1H NMR chemical shifts of compounds **2** and **4** ($CDCl_3$)

	2	**4**
Me-18	0.86	0.86
Me-19	1.04	1.04
Me-21	1.29	1.26
Me-26	1.24	1.21
Me-27	1.24	1.23
H-2	5.04	5.04
H-3	5.32	5.31
H-7	5.84	5.85
H-9	3.10	3.10
H-22	—	4.79
OAc	2.01	1.99
	2.12	2.10
		2.10

Table 2. ^{13}C NMR chemical shifts of compounds **2** and **4** ($CDCl_3$)

Carbon No.	2	4
1	38.4 (*t*)	38.4 (*t*)
2	67.0 (*d*)	67.2 (*d*)
3	68.7 (*d*)	68.7 (*d*)
4	34.0 (*t*)	34.1 (*t*)
5	51.0 (*d*)	51.0 (*d*)
6	202.0 (*s*)	202.2 (*s*)
7	121.5 (*d*)	121.6 (*d*)
8	164.8 (*s*)	164.8 (*s*)
9	33.6 (*d*)	33.7 (*d*)
10	38.4 (*s*)	38.4 (*s*)
11	20.4 (*t*)	20.5 (*t*)
12	31.5 (*t*)	31.2 (*t*)
13	46.9 (*s*)	47.6 (*s*)
14	84.8 (*s*)	84.4 (*s*)
15	30.9 (*t*)	31.4 (*t*)
16	29.7 (*t*)	29.2 (*t*)
17	52.2 (*d*)	49.6 (*d*)
18	17.5 (*q*)	17.5 (*q*)
19	23.8 (*q*)	23.8 (*q*)
20	75.0 (*s*)	77.0 (*s*)
21	26.5 (*q*)	20.5 (*q*)
22	44.3 (*t*)	79.9 (*d*)
23	18.7 (*t*)	24.8 (*t*)
24	44.7 (*t*)	40.4 (*t*)
25	71.0 (*s*)	70.5 (*s*)
26	29.3 (*q*)	28.5 (*q*)
27	29.5 (*q*)	30.3 (*q*)

Compound **1** exhibited a potential moulting effect although slightly less than that of ecdysterone (**3**) as listed in Table 3. Thus, it is conceivable that the presence of the 22*R*-hydroxyl is required for expression of high moulting activity.



1 R = R' = H
2 R = Ac, R' = H
3 R = H, R' = OH
4 R = Ac, R' = OAc

EXPERIMENTAL

Extraction and isolation. Commercial dried plant material (300 g) was extracted with refluxing MeOH. The MeOH extract was evaporated *in vacuo* to dryness and the residue (60 g) was partitioned between *n*-BuOH and H_2O (1:1). The organic layer was evaporated *in vacuo* and the residue (29 g) was defatted with *n*-hexane. The residue (22 g) was chromatographed on Si gel ($CHCl_3$–MeOH, 20:1) to give fractions 1–12. Fraction 6 was acetylated with Ac_2O–pyridine at room temp. overnight in order to achieve more facile separation to afford the crude acetate of **1**. Further repeated CC using Si gel (*n*-hexane–Me_2CO, 3:1; $CHCl_3$–MeOH, 100:1) afforded **2** (26 mg), $[\alpha]_D^{22}$ +45.0° ($CHCl_3$; *c* 1.00), R_f 0.39 on Si gel TLC $CHCl_3$–MeOH (10:1); 0.49 (ecdysterone 2, 3, 22-triacetate, **4**), MS *m/z*: 512[M − $2H_2O$]$^+$, 494[M − $3H_2O$]$^+$, 451, 385, 283, 108.

Saponification of **2**. Alkaline treatment of **2** (26 mg) with 3% K_2CO_3–MeOH at room temp. for 30 min, neutralization with weak acid and successive desalting using Sephadex LH-20 with MeOH afforded **1** (11 mg), an amorphous powder (mp 120–125°), $[\alpha]_D^{29}$ + 71.9° (MeOH; *c* 0.57), R_f 0.35 on Si gel TLC $CHCl_3$–MeOH–H_2O, (8:2:0.2); 0.30 (ecdysterone), IR ν_{max}^{KBr} cm^{-1}: 3400(OH), 2950, 1650(enone), MS *m/z*: 446[M − H_2O]$^+$, 428[M − $2H_2O$]$^+$, 410[M − $3H_2O$]$^+$, 392[M − $4H_2O$]$^+$, 377, 349, 309, 283, 109 (base peak, $C_8H_{13}^+$), the

Table 3. Effect of taxisterone (**1**) and ecdysterone (**3**) on silkworm growth

Compound	Stage*	Number of silkworms						Remarks
		1 day	2 days	3 days	4 days	5 days	6 days	
Ecdysterone	L	1	—	0	—	—	—	
	M	4	—	0	—	—	—	
	P	0	—	5	—	—	—	
Taxisterone	L	5	—	0	0	—	—	
	M	0	—	3	0	—	—	10 μg/larva (5th instar)
	P	0	—	2	5	—	—	(inject to abdomen)
Control	L	5	—	5	5	0	0	
	M	0	—	0	0	5	0	
	P	0	—	0	0	0	5	
Ecdysterone	L	—	0	—	—	—	—	
	M	—	0	—	—	—	—	
	P	—	5	—	—	—	—	
Taxisterone	L	—	0	0	—	—	—	
	M	—	4	0	—	—	—	10 μg/larva (5th instar)
	P	—	1	5	—	—	—	(oral)
Control	L	—	5	5	0	0	—	
	M	—	0	0	5	0	—	
	P	—	0	0	0	5	—	

*L, Larva; M, moulting stage; P, pupa.

fragments usually observed at *m/z* 99 and 81 due to cleavage at C-20, C-22 were not detected.

Since **2** on weak base treatment regenerated the acetyl-free compound **1**, whose R_f value on TLC was coincident with that of the compound in the original methanolic extractive, it was considered that **1** probably exists in an acetyl-free form in the plant material.

Moulting test of **1**. A suspension of **1** in H_2O (2 mg/ml) was used as a test sample. An aq. soln of **3** (2 mg/ml, Rohto Pharmaceutical Co. Ltd.) for reference and H_2O for control were used. Each soln was injected directly into the centre of the abdomen or administered orally into the alimentary canal, using a micro-syringe at a dose of 10 μg (0.005 ml)/larva (5th instar), respectively.

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TRACE FORMATION OF PROGESTERONE FROM 22-DEHYDRO PHYTOSTEROLS DURING STORAGE

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Key Word Index—Progesterone; 22-dehydro phytosterols; side chain oxidation; storage.

Abstract—Progesterone has been isolated from different samples of 22-dehydro phytosterols of previously known composition, kept in a laboratory collection for periods from 10 to 18 years. It is not present in detectable amount in cholesterol, fucosterol or desmosterol, or in freshly prepared samples of stigmasterol. The possibility of progesterone being an autoxidation artifact is thus emphasized, in particular in the environment, and hence, in concentrating organisms.

During the course of recrystallization of sterol samples of previously known composition which had been kept in the laboratory collection for several years, we observed strongly UV absorbing substances accumulating in the mother liquors. A substance having the same R_f as progesterone was detected in the case of old samples of 22-dehydro sterols while the corresponding spot was absent in samples of other sterols. A progesterone fraction was isolated from an 18 years old sample of stigmasterol of known composition (checked by MS, TLC, GC) after two successive chromatographies of the methanol mother liquors on Si gel. This product had the expected molecular ion for progesterone at *m/z* 314 with the characteristic [1] fragments at *m/z* 43 $[MeCO]^+$, 272 ($[M-42]^+$, elimination of ketene from the 3-oxo 4-ene conjugated system) and 299 $[M-15]^+$. A second molecular ion at *m/z* 316 of nearly equal intensity indicated a derivative which, due to scarcity of the isolated fraction, could not be separated. The UV spectrum (240 nm, ethanol) was in agreement with an α, β-unsaturated carbonyl group. A 2, 4-dinitrophenylhydrazone was prepared from a methanolic solution and submitted to re-isolation by TLC. The product showed the same R_f as an authentic sample of progesterone-2, 4-DNP, and gave a mass spectrum with the molecular ion at *m/z* 674 for a bis-2, 4-DNP.

A systematic investigation was performed on the other sterol samples in the collection, and progesterone was found in all the aged 22-dehydro phytosterol-containing products. Progesterone was also isolated from several pollen sterols of reported composition [2], together with a series of UV absorbing products which have not been further investigated due to scarcity. Thus, when originally isolated in 1967 [2] from a mustard pollen (*Brassica nigra*), a sterol fraction contained 37% methylene cholesterol, 9% mono-unsaturated C_{27} sterol, 32% di-unsaturated C_{29} sterol and 7% mono-unsaturated C_{29} sterol. After recrystallization in methanol, the relative concentrations have now changed to 10% for the C_{28} and