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ECDYSTEROIDS OF THE ROOT BARK OF *VITEX CANESCENS*

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Key Word Index—*Vitex canescens*; Verbenaceae; root bark; ecdysteroids; 24-*epi*-abutasterone.

Abstract—A new ecdysteroid, 24-*epi*-abutasterone, was isolated from the root bark of *Vitex canescens*. 20-Hydroxyecdysone, 24-*epi*-makisterone A, shidasterone, calonysterone and turkesterone were also isolated from this plant species. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Among the *Vitex* plants reported to be endemic to Thailand, barks of *V. glabrata*, *V. pinnata* and *V. canescens* have been reported to contain 20-hydroxyecdysone (**1**) and turkesterone (**2**) as major ecdysteroids [1–3]. Pinnatasterone [2] and canescensterone [3] have been isolated as minor ecdysteroids of the second and third *Vitex* species, respectively. The occurrence of calonysterone (**3**) in the first plant species has also been mentioned [4]. We now report the isolation of a number of ecdysteroids from the root bark of *V. canescens* and, among them, 24-*epi*-abutasterone (**4**) which is a new ecdysteroid.

RESULTS AND DISCUSSION

The crude ethanol extract of the root bark of *V. canescens* revealed a more complex mixture than those obtained from the stem barks of this plant species [3] and other *Vitex* species [2]. 20-Hydroxyecdysone (**1**) was the major ecdysteroid isolated from both the chloroform and ethyl acetate extracts. 24-*epi*-Makisterone A (**5**) [5, 6] was isolated as a minor ecdysteroid from the former extract, whereas shidasterone (**6**) [7, 8], calonysterone (**3**) [4, 9] and turkesterone (**2**) [1] were the minor ecdysteroids isolated from the latter extract. The ecdysteroid **6** was also present in the chloroform extract. The ^{1}H NMR data of **5** (Table 1) were consistent with the structure and the chemical shift values of the methyl resonances were in agreement with those reported previously [5]. The ^{13}C NMR data of **5** (not shown) were also consistent with the reported values [5]. The identity of compounds **1**–**3** and **6** was established by comparisons with the spectroscopic values and, in some cases, was confirmed by TLC comparisons with authentic samples (see Experimental). It should be noted that the configuration at C-22 of compound **6** has recently been established as 22*R* [8].

A new ecdysteroid, 24-*epi*-abutasterone (**4**), was isolated as another minor component from the ethyl acetate extract. High resolution FAB-mass spectrum established a molecular formula of $C_{27}H_{44}O_8$. The ^{1}H NMR spectrum (Table 1) of this compound was very similar to that of abutasterone (**7**) [10, 11]. The spectral features and relative positions of H-2, H-3, H-5, H-7, H-9 and H-17, as well as those of 18-Me and 19-Me indicated that these two ecdysteroids possessed the same nucleus. The two *dd* signals at δ 4.51 (J = 9.6 and 2.3 Hz) and 4.37 (J = 9 and 2.7 Hz) indicated that the two side-chain hydroxyl groups should be located at C-22 and C-24. Another side-chain hydroxyl group should be located at C-25, since the C-26 and C-27 resonances appeared as two singlet signals at δ 1.46 and 1.47. It was thus evident that the oxygenation pattern of the new ecdysteroid and compound **7** were the same, but differing in stereochemical arrangement of some hydroxyl group. The chemical shift value of the 21-Me of this ecdysteroid and compound **7** were of the same magnitude, suggesting the spatial arrangement of the hydroxyl groups at C-20 and C-22 to be the same. The structure of **4** was further confirmed by ^{13}C NMR spectral comparison with that of compound **7** [11] (Table 2). The only significant differences between the chemical shift values of the ^{13}C resonances of these two compounds were those of C-22, C-23 and C-24 (see Table 2). The observations were in agreement with those for pterosterone [12] and 24-*epi*-pterosterone [13], where the C-22, C-23 and C-24 resonances of the former appeared at δ77.4, 35.7 and 76.7, while those of the latter appeared at δ73.4, 37.5 and 73.5. It was thus

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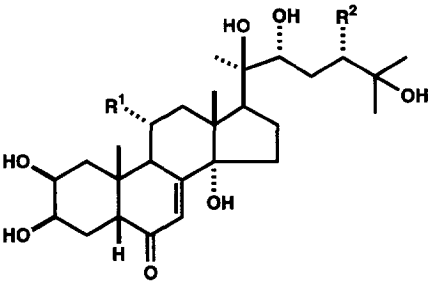
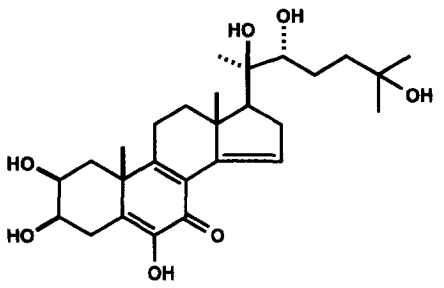


1 $R^1 = R^2 = H$
2 $R^1 = OH, R^2 = H$
5 $R^1 = H, R^2 = Me$
7 $R^1 = H, R^2 = OH$



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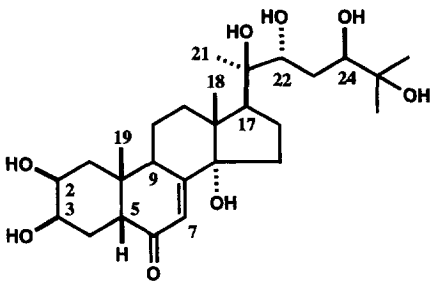

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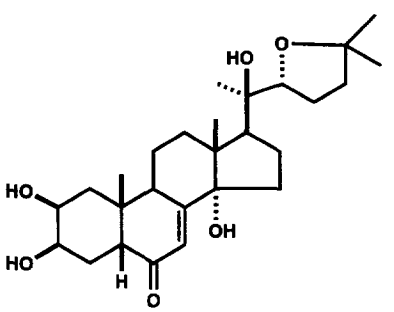

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concluded that the new ecdysteroid differed from **7** only in the configuration of the hydroxyl group at C-24. The structure of the new ecdysteroid was therefore established as 24-*epi*-abutasterone (**4**).

EXPERIMENTAL

General. ^1H and ^{13}C NMR were recorded at 500 and 125.65 MHz, respectively. Unless indicated otherwise, Merck silica gel 60 (>230 mesh) was used for CC. TLC was conducted on plates precoated with Merck silica gel 60 F_{254}. Spots on TLC were visualized under UV light and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

Plant material. Root bark of *V. canescens* was collected in Pipoon District, Nakhon Si Thammarat Province. A voucher specimen of this plant is deposited at the Plant Collection Centre, Department of Biology, Ramkhamhaeng University.

Extraction and isolation. Pulverized, dry root bark (2.5 kg) was extracted successively with *n*-hexane and EtOH in a Soxhlet extraction apparatus. The concd EtOH extract was diluted with H_2O and the filtered dark brownish soln extracted successively with $CHCl_3$ and EtOAc, using a continuous liquid–liquid extraction apparatus. The $CHCl_3$ extract (100.0 g) was chromatographed (Merck silica gel 60, 70–230 mesh) using $CHCl_3$–MeOH as eluent, with increasing MeOH content, and two main frs were selected by TLC examination of the eluates. The first fr. (fr. C1) was repeatedly chromatographed to afford 7 mg of 24-*epi*-makisterone A (**5**). The ^1H NMR data are given in Table 1. The ^{13}C NMR data were consistent with the reported information [5]. The second fr. (fr. C2) was rechromatographed and crystallized from MeOH–EtOAc to give 20-hydroxyecdysone (**1**) (5.36 g). TLC comparison with authentic sample [1], and IR and ^1H NMR data were consistent with those reported for **1** [1].

The EtOAc extract (46.9 g) was subjected to CC (Merck silica gel 60, 70–230 mesh), using a similar eluting solvent as that employed for the $CHCl_3$ extract, and four selected frs (fr. E1–E4) were subjected to further investigation/sepn. Fr. E1, eluted by $CHCl_3$–MeOH (88:12 to 86:14), was rechromatographed to give crude ecdysteroid fr. (52 mg), which was again rechromatographed and the resulting ecdysteroid-containing fr. was further sepd and purified by HPLC [column: Spherisorb ODS2, 5 μm, 250 × 4.6 mm; mobile phase: MeOH–H_2O (1:1); flow rate: 1.0 ml min^{-1}, detector: 254 nm] to give 5 mg of shidasterone (**6**). It should be noted that reinvestigation of a $CHCl_3$ fr. revealed the presence of compound **6** among a complex mixt. of non-ecdysteroid components, but no further isolation of this ecdysteroid was made.

Fr. E2, eluted by $CHCl_3$–MeOH (84:16 to 82:18), constituted a number of components and colour reactions of some of these components on TLC were indicative of ecdysteroids. After three repeated CC, two subfrs (subfr. 1 and subfr. 2) were selected for further purification by HPLC (column: μBondapak C18, 10 μm, 300 × 3.9 mm; mobile phase: MeOH–H_2O (1:1); flow rate: 1.0 ml min^{-1}; detector: 254 nm). From subfr. 1, more compound **6** (3 mg) was isolated. From subfr. 2, calonysterone (**3**) (2 mg) was isolated and identified by TLC and spectroscopic (IR and ^1H NMR) comparisons with those of authentic **3** isolated from the bark of *V. glabrata* [4].

Table 1. ^{1}H NMR data of compounds **4**, **5** and **7**

H	4	5	7*
2	4.18 (*m*)	4.18 (*m*)	4.20
3	4.23 (*br s*)	4.23 (*br s*)	4.22
5	2.99 (*dd*, 12.9, 3.5)	3.01 (*dd*, 13.1, 3.6)	3.01 (*dd*, 15, 3.8)
7	6.22 (*d*, 2.1)	6.26 (*d*, 2.7)	6.24 (*d*, 2.2)
9	3.57 (*m*)	3.59 (*m*)	3.59 (*m*)
17	3.08 (*t*, 9.1)	3.00 (*t*, 9.1)	2.98 (*t*, 8.4)
22	4.51 (*dd*, 9.6, 2.3)[a]	4.07 (*br d*, 9.4)	4.25
24	4.37 (*dd*, 9, 2.7)[a]		4.08 (*d*, 9.1)
18-Me	1.22 (*s*)	1.21 (*s*)	1.20 (*s*)
19-Me	1.06 (*s*)	1.06 (*s*)	1.07 (*s*)
21-Me	1.63 (*s*)	1.58 (*s*)	1.61 (*s*)
26-Me	1.46 (*s*)	1.33 (*s*)	1.47 (*s*)[b]
27-Me	1.47 (*s*)	1.38 (*s*)	1.52 (*s*)[b]

All compounds were recorded in pyridine-d_5.
* Data taken from ref. [11].
[a,b] Assignments may be reversed for signals with the same superscript.

Table 2. ^{13}C NMR data of compounds **4** and **7**

C	4	7*
1	37.9	37.9
2	68.1[a]	68.0[c]
3	68.0[a]	67.9[c]
4	32.3	32.4
5	51.3	51.3
6	203.4	203.4
7	121.6	121.6
8	166.1	166.0
9	34.4	34.4
10	38.6	38.6
11	21.1	21.0
12	31.7	31.6
13	48.1	48.0
14	84.2	84.0
15	31.9	31.9
16	21.3	21.4
17	50.0	49.9
18	17.9	17.8
19	24.4	24.4
20	76.8	76.6
21	21.7	21.7
22	73.7[b]	78.1
23	35.2	32.8
24	75.9[b]	80.2
25	72.6	72.1
26	26.1	26.8[d]
27	26.0	25.1[d]

All compounds were recorded in pyridine-d_5.
* Data taken from ref. [11].
[a–d] Assignments may be reversed for signals with the same superscript.

Fr. E3 constituted mainly the ecdysteroid **1**, isolated as a major component from the $CHCl_3$ extract, but it was contaminated by a number of minor components.

Fr. E4, eluted by $CHCl_3$–MeOH (76:24 to 72:28), was subjected to further sepn by HPLC (Delta Pak C18, 15 μm, 300 × 7.8 mm, mobile phase: MeOH–H_2O (7:15); flow rate: 3.0 ml min^{-1}; detector: 254 nm) to give three subfrs. The first subfr. contained turkesterone (**2**) (4 mg from 1/3 of the crude subfr.). The identity of **2** was made by TLC comparison with an authentic sample [1] and agreement of ^{1}H NMR data with those reported previously [1]. The third subfr was shown to be more of the ecdysteroid **1**.

TLC multiple development ($CHCl_3$–MeOH, 5:1, 3 runs) of the second subfr. revealed two components. Repeated HPLC of this subfr [Spherisorb ODS2, 5 μm, 250 × 4.6 mm; mobile phase: MeOH–H_2O (1:3); flow rate: 1.0 ml min^{-1}; detector: 254 nm] resulted in the sepn of two ecdysteroids. The first component (3 mg) was a new ecdysteroid, 24-*epi*-abutasterone (**4**). The second component (*ca* 0.5 mg) was an unidentified ecdysteroid.

24-epi-*Abutasterone* (**4**). IR ν_{max}^{KBr} cm^{-1}: 3408, 2962, 1651, 1444, 1383, 1303, 1057, 950, 877. ^{1}H and ^{13}C NMR data are given in Table 1 and 2, respectively. HR-FABMS *m/z*: 497.3123 $[M+H]^+$ (calc. for $C_{27}H_{45}O_8$: 497.3114).

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