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AROMATIC AND OTHER CONSTITUENTS OF FOUR *VERBESINA* SPECIES: STRUCTURE AND STEREOCHEMISTRY OF VERBESINDIOL*

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Key Word Index—*Verbesina sublobata*; *V. gigantea*; *V. myriocephala*; *V. virginica*, Compositae; 2,6-dimethoxybenzoquinone; syringaldehyde; syringic acid; eudesmic acid; euparin; coumaric acid; 6-*p*-coumaryl ester of verbesindiol; eudesmane derivative.

Abstract—Chemical examination of *Verbesina sublobata*, *V. gigantea*, *V. myriocephala* and *V. virginica* yielded mainly aromatic aldehydes and acids along with 2,6-dimethoxybenzoquinone and euparin as colouring matters. *V. virginica* also gave the *p*-coumarate ester of a new sesquiterpenediol, verbesindiol, related to the verbesinols whose structure and stereochemistry were elucidated.

INTRODUCTION

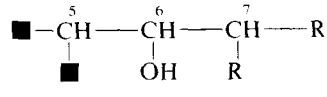
Relatively few members of the large genus *Verbesina* (Heliantheae, Compositae) have been studied chemically; the results present a somewhat confusing picture. While two Mexican species have yielded sesquiterpene lactones of the elemanolide group [1–3], cinnamate esters of various types, including cinnamates of eudesmane alcohols, seem to be characteristic constituents of some other species, especially their roots [4–8]. Other constituents seem not particularly distinctive [9–11]. We now report the results of a chemical investigation of four *Verbesina* species which fit in with the earlier work.

RESULTS AND DISCUSSION

The herbaceous parts of *Verbesina sublobata* Benth. contained in addition to 2,6-dimethoxybenzoquinone (**1**) syringaldehyde (**2a**), syringic acid (**2b**) and eudesmic acid (**2c**). *V. gigantea* Jacq. gave the same quinone, but different acids, namely 3,4-dimethoxycinnamic acid (**3b**) and ferulic acid (**3a**). *V. myriocephala* Sch. Bip. whose polar constituents had been reported previously [11] contained sitosterol, **2b**, **2c** and **3a**, whereas euparin (**4**), **1**, *p*-coumaric acid and the *p*-coumaryl ester **5a** of a new eudesmanediol **5b**, which we have named verbesindiol, were found in the herbaceous parts of *V. virginica* L.† Details of the structure elucidation of **5a** are presented below.

The new substance, $C_{24}H_{34}O_4$ (high resolution MS), was a non-crystallizable gum and had spectral properties appropriate for the *p*-coumarate ester of a sesquiterpene diol (MS base peak $C_9H_8O_3$, IR bands at 3350, 1690, 1605, 1592 and 840 cm^{-1}, ^1H NMR signals in Table 1, ^{13}C NMR signals in Table 2). Alkaline hydrolysis gave *p*-coumaric acid and verbesindiol **5b**, $C_{15}H_{16}O_2$, in whose NMR spectrum the H-6 signal (numbering as in finally-deduced structure) had experienced the expected upfield shift from 5.86 to 4.20 ppm. The corresponding carbon doublet had also shifted upfield, from 69.28 to 66.32 ppm, whereas a singlet near 73 ppm (C-4) indicating the location of a tertiary hydroxyl group, had remained unaffected during the hydrolysis. Other characteristic ^1H NMR signals included those of an isopropyl group (two methyl doublets at 0.96 and 0.93 ppm which collapsed on irradiation in the methylene and methinyl envelope, see Table 1), and methyl singlets at 1.16 and 1.50 ppm. The chemical shift of the latter indicated that it was attached to carbon carrying the tertiary hydroxyl group; the significance of its paramagnetic shift in the hydrolysis of **5a** to **5b** will be commented upon subsequently in connection with the stereochemical discussion.

Oxidation of the diol furnished a ketol **6a** whose spectral properties (new IR band at 1692 cm^{-1}, carbonyl singlet at 213 ppm) indicated that the new ketone group was not that of a cyclopentanone. This and the demonstration by spin decoupling of the presence of partial structure A where $J_{5,6} = 2.5$, $J_{6,7} < 2$ Hz strongly suggested that the new substance was a hydrated form **5a** of two verbesinol esters from the roots of *V. virginica* [4] to which the relative stereochemistry shown in formulae **7a** and **7b** rather than the one originally assigned [4] was ascribed while our work was in progress because of the small values of $J_{5,6}$ and $J_{6,7}$ [5]. However the reported data do not necessarily exclude other stereochemical possibilities and the absolute configuration is unknown.



A

Oxidation of **5b** to **6a** was accompanied by an upfield shift of one of the methyl singlets ($\Delta\delta = 0.29$ ppm) which suggested that the C-6 hydroxyl and the C-10 methyl of **5a**

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† The roots of this species were studied earlier [4, 5].

Table 1. ^{1}H-NMR spectra*

	5a†	5b	5b‡	5b§	6a	6a‖	6a§	8
H-5	1.43*dbr* (2.5)	1.10*dbr*	2.07*dbr*	1.46*dbr*	2.30*br*	2.19*br*	2.58	—
H-6	5.86*br* ($W_{\frac{1}{2}}$ = 8)	4.20*br*	5.92*br*	4.90*br*	...	...	...	6.14*br*
H-7	•	•	•	1.60•	•	•	•	...
H-11	1.43•	•	•	1.58•	2.27*m*	2.31*m*	2.25*m*	2.30*sept* (7)
H-12	0.90*d* (7)	0.96*d*	1.03*d*	1.00*d*	0.90*d*	0.86*d*	0.89*d*	1.07*d*
H-13	0.90*d* (7)	0.93*d*	1.00*d*	0.96	0.83*d*	0.84*d*	0.88*d*	1.06*d*
H-14	1.16	1.16	1.54	1.52	0.87	0.62	0.82	0.95
H-15	1.19	1.50	2.30	1.92	1.47	1.55	1.74	1.70

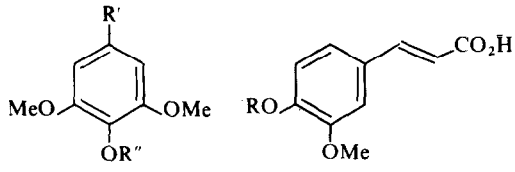
* Run at 270 MHz in $CDCl_3$ unless specified otherwise. Unmarked signals are singlets. Coupling constants in parentheses in hertz.
† Other signals 6.29*d* (16, H-2'), 6.87*d* (8.5, H-5', 9'), 7.43*d* (8.5, H-6', H-8'), 7.65*d* (16, H-3').
‡ In $CDCl_3$ + 0.25 mol equiv. Eu(fod)$_3$.
§ In C_5D_5N.
‖ In C_6D_6.
• Overlapping or obscured signal.

Table 2. ^{13}C NMR spectra*

Carbon	5a	5b	6a
1	45.18*t*†	45.32*t*†	43.08*t*†
2	19.93*t*	20.30*t*	19.68*t*
3	43.68*t*†	43.90†	40.59*t*†
4	72.83	72.95	71.00
5	56.84*d*	57.68*d*	66.66*d*
6	69.28*d*	66.32*d*	213.28
7	50.00*d*	50.85*d*	55.96*d*
8	21.40*t*	21.10*t*	22.29*t*
9	43.13*t*†	45.32†	41.03*t*†
10	34.86	34.89	39.75
11	28.73	28.84*d*	25.62*d*
12	21.23*q*	20.84*q*	20.70*q*
13	21.23*q*	20.79*q*	20.70*q*
14	20.69*q*	21.65*q*	20.38*q*
15	24.45*q*	25.11*q*	24.09*q*
1'	169.22		
2'	114.56*d*		
3'	146.13*d*		
4'	125.94		
5', 9'	130.21*d*		
6', 8'	116.10*d*		
7'	159.28		

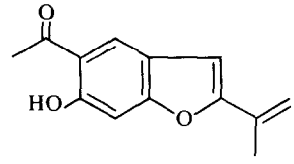
* Run in $CDCl_3$ at 67.9 MHz. Unmarked signals are singlets.
† Assignments may be interchanged.

were *cis* and axial. Analogously the downfield shift of the second methyl singlet accompanying the conversion of **5a** to **5b** ($\Delta\delta$ = 0.34 ppm) and failure of the latter to form an acetonide indicated that the C-4 methyl group was also axial. These conclusions were supported by the Eu(fod)$_3$-induced shifts of the two methyl groups in **5b** reported in Table 1 and the demonstration of an appreciable NOE (15%) between the two methyl groups of **6a**. Additionally, the downfield appearance of H-11 of **6a**, not visible in the NMR spectra of **5a** and **5b**, at 2.27 ppm indicated that the C-7, C-11 bond was parallel or nearly so to the plane of the C-6 carbonyl and hence equatorial; in fact, the chemical shifts of **6a** closely resemble those reported for ketol **6b** from pygmol [12]. Hence the relative sterochemistry is that shown in formulae **5a**, **5b** and **6a**; this is further supported by (1) the benzene-induced shifts in the NMR spectrum of **6a** which demand that the C-10 methyl lie behind the plane of the benzene · ketol collision complex, the C-4 methyl and H-11 very near the plane but on the front, toward oxygen, and H-5 between the C-10 methyl



2a R′ = CHO, R″ = H
2b R′ = CO_2H, R″ = H
2c R′ = CO_2H, R″ = OMe

3a R = Me
3b R = H



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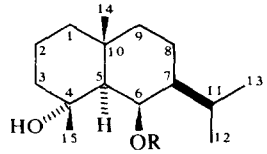
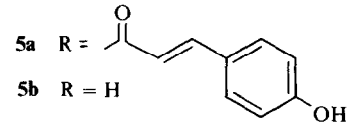
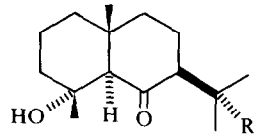
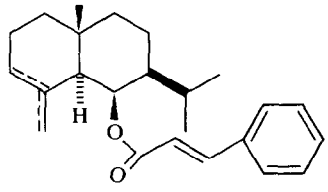




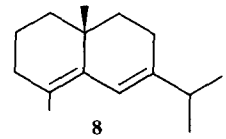
5a R =
5b R = H



6a R = H
6b R = OH



7a 3,4-*db*
7b 4,15-*db*



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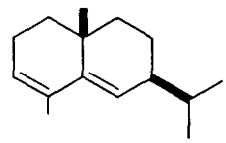

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and the plane, and (2) the pyridine-induced shifts in the NMR spectra of **5b** and **6a***.

With the relative configuration established, the positive Cotton effect curve of ketol **6a** pointed to the absolute configuration depicted in the formulae. This was confirmed as follows. Treatment of **5a** with $POCl_3$–Py afforded two isomeric dienes **8** and **9** in a 4:1 ratio. The major diene had a positive rotation and exhibited the strongly positive Cotton effect of (+)-δ-selinene of established absolute configuration (C-10 methyl β) [14, 15]. The CD curve of the minor diene **9**, necessarily with the same C-10 stereochemistry, displayed the negative Cotton effect of (−)-selina-3,5-diene, also of established absolute configuration [15]. Consequently, the new sesquiterpene ester is the 6-*p*-coumaryl ester of (4*R*,5*S*,6*R*,7*S*,10*R*) 4,6-eudesmanediol. We assume that the verbesinol esters **7a**, **b** from the roots of our species [4, 5] possess the same absolute configuration, but it is interesting to note that the rupestrol esters from *V. rupestris* are said to belong to the *enantio*-eudesmane series [6, 7].

* In saturated cyclic systems, pyridine deshields protons and methyls which are 1,3-diaxial, vicinal or germinal to hydroxyl. Maximum deshielding of vicinal protons is produced for a dihedral angle of 60° [13].

EXPERIMENTAL

Extraction of Verbesina sublobata. Above-ground parts of *V. sublobata* Benth., collected by G. Cruz in 1975 near Tegucigalpa, Hondurus, wt 12 kg (voucher on deposit in herbarium of UNAH, accession no. P.R. 80869 of Medicinal Plant Resources Laboratory, USDA) were extracted with $CHCl_3$ and worked up in the usual way [16]. The crude gum, wt 23 g, was chromatographed over 300 g of silicic acid (Mallinckrodt 100 mesh), 200 ml fractions being eluted in the following order: fractions 1–5, toluene; 5–10 tol–$CHCl_3$ (1:1); 11–16 $CHCl_3$; 17–21 $CHCl_3$–MeOH (99:1); 22–26 $CHCl_3$–MeOH (97:3); 27–31 $CHCl_3$–MeOH (19:1) and 32–38 $CHCl_3$–MeOH (10:1). Fraction 11–16 gave 0.1 g of yellow 2,6-dimethoxybenzoquinone (**1**), mp 248°. Fraction 17–21 contained syringaldehyde (**2a**) as major constituent which was purified by prep. TLC ($CHCl_3$–MeOH, 9:1) and recrystallized from $CHCl_3$–hexane, yield 0.1 g, mp 110–112°. Fractions 22–26 showed one major spot on TLC; recrystallization afforded 50 mg of eudesmic acid (**2c**), mp 168°. The major constituent of fractions 32–38 was purified by prep. TLC ($CHCl_3$–MeOH, 17:3), recrystallized from $CHCl_3$–hexane and identified as syringic acid (**2b**), mp 200°, yield 0.5 g.

Extraction of V. gigantea. Above-ground parts of *V. gigantea* Jacq., collected by G. Cruz near Tegucigalpa, Honduras in 1974, wt 6 kg and were extracted with $CHCl_3$. The crude gum, wt 10 g,

was chromatographed over 100 g of silicic acid, 100 ml fractions of eluate being collected as follows: fractions 1–5 $CHCl_3$–tol (1:1), fractions 6–10 $CHCl_3$, fractions 11–16 CHl_3–MeOH (99:1), 17–21 $CHCl_3$–MeOH (97:3), 22–25 $CHCl_3$–MeOH (19:1) and 26–30 $CHCl_3$–MeOH (10:1). Fractions 11–16 on crystallization from $CHCl_3$–hexane furnished 50 mg of **1**. Fractions 17–21, purified by prep. TLC ($CHCl_3$–MeOH, 9:1) and crystallization from $CHCl_3$–hexane gave 50 mg 3,4-dimethoxycinnamic acid (**3a**), mp 177°. Fractions 22–25 were separated by prep. TLC into additional **3a** and 70 mg ferulic acid (**3b**) mp 167–169°.

Extraction of V. myriocephala. Above-ground parts of *V. myriocephala* Sch. Bip., wt 5.8 kg, collected by R. Lazor on December 18, 1971 near Cerro Campana, Province, Panama (voucher Lazor No. 5808 on deposit in herbarium of Florida State University) were extracted with $CHCl_3$ and worked up as usual. The isolation of rhamnocitrin-3-glucuronide from the methanol extract of this collection has been described [11]. The crude gum from the $CHCl_3$ extract, wt 16.5 g, was absorbed on 20 g of silicic acid and chromatographed over 200 g of silicic acid. 200 ml fractions being collected in the following order: fractions 1–5 $CHCl_3$, 6–10 ($CHCl_3$–MeOH (99:1), 11–16 $CHCl_3$–MeOH (97:3), 17–21 $CHCl_3$–MeOH (19:1) and 22–25 $CHCl_3$–MeOH (10:1). Fractions 6–10 gave 50 mg of sitosterol, fractions 11–16 gave 20 mg of pure **2c**, fractions 17–21 gave 20 mg of pure **2b** and fractions 22–25 gave 10 mg of pure **3b**.

Extraction of V. virginica. Above-ground parts of *V. virginica* L., wt 3 kg, collected by Dr. B. H. Braun in the vicinity of Kansas City, Mo. in summer 1959, were extracted with $CHCl_3$. The usual work-up gave 5 g of crude gum which was chromatographed over 150 g of silicic acid and eluted in 100 ml fractions. Fractions 1–5 ($CHCl_3$) gave after crystallization 10 mg of **1**. Fractions 6–10 ($CHCl_3$–MeOH, 99:1) after purification by prep. TLC and recrystallization from EtOH gave 50 mg of euparin (**4**), mp 120–122°. Fractions 11–14 ($CHCl_3$–MeOH, 97:3) contained one major constituent which was purified by prep. TLC and could not be induced to crystallize, yield of **5a** 0.5 g, $[\alpha]_D$ – 23° (*c* 0.50, $CHCl_3$), IR (KBr) 3350 (*br*), 1690, 1635, 1605, 1592, 1270 and 840 cm^{-1}. (Calculated for $C_{24}H_{34}O_4$: MW, 386.2457. Found: MW(MS), 386.2449). Other prominent ions in the MS were found at 371, 368, 353, 222, 204, 189, 164 ($C_9H_8O_3$, base peak) and 147.

A solution of 0.25 g of **5a** in 5 ml of EtOH and 0.2 g of KOH in 2 ml of H_2O was refluxed for 2 hr, neutralized with HOAc, diluted with H_2O and extracted with EtOAc. The washed and dried organic layer gave a gum which was purified by prep. TLC. Recrystallization from $CHCl_3$–hexane afforded 0.14 g **5b**, mp 96–97°, $[\alpha]_D$ – 35° (*c* 0.25, $CHCl_3$), IR (KBr) 3420, 1465, 1380, 1370, 1030 and 910 cm^{-1}. The high resolution MS did not show the molecular ion but had significant peaks at *m/e* (composition, %) 222 ($C_{15}H_{26}O$, 24.1), 208 ($C_{14}H_{24}O$, 28.3), 207 ($C_{14}H_{23}O$, 100), 204 ($C_{15}H_{24}$, 13), 190 ($C_{14}H_{22}$, 4), 189 ($C_{14}H_{21}$, 28.4), 180 ($C_{12}H_{20}O$, 5.7), 179 ($C_{12}H_{19}O$, 14), 164 ($C_{12}H_{20}$, 6), 163 ($C_{12}H_{19}$, 4), 162 ($C_{12}H_{18}$, 6.3), 161 ($C_{12}H_{17}$, 35), 154 ($C_{10}H_{18}O$, 11.2), 153 ($C_{10}H_{17}O$, 85.9), 149 ($C_{11}H_{17}$, 35), 148 ($C_{11}H_{16}$, 4.7), 147 ($C_{11}H_{15}$, 14.5), 139 ($C_9H_{15}O$, 7.3), 138 ($C_9H_{14}O$, 4.5), 138 ($C_{10}H_{18}$, 5.1), 137 ($C_{10}H_{12}$, 48.6), 137 ($C_9H_{13}O$, 21.9), 136 ($C_{10}H_{16}$, 8.5), 135 ($C_{10}H_{15}$, 9.5), 134 ($C_{10}H_{14}$, 6) and 133 ($C_{10}H_{13}$, 19.4). A negative ion CI MS (CH_2Cl_2) showed two prominent ions at 275 $[(M + Cl)^-]$ and 257 $[(M - 18 + Cl)^-]$.

A solution of 50 mg **5b** in 5 ml of ether was oxidized with CrO_3 using procedure B of Brown *et al.* [17]. Work-up and prep. TLC gave 45 mg **6a** which could not be induced to crystallize. IR (neat) 3500 (broad), 1092, 1390, 1375, 1190, 1130 and 900 cm^{-1}. CD curve (MeOH) $[\theta]_{283}$ + 2100. MS 238 (M^+), 223, 220, 205, 178, 135 (base peak), 109, 107 and 93.

Exposure of 80 mg of **5a** to $POCl_3$–Py for 15 min and the usual work-up gave a mixture of two non-polar substances (NMR, TLC) which were separated by prep. TLC (hexane, 3 developments). The upper band gave 5 mg **9** as a colourless oil, CD curve (MeOH) $[\theta]_{230}$ – 40300. MS 204 (M^+). The lower band gave 20 mg of **8**, $[\alpha]_D$ + 269° (*c* 0.20, $CHCl_3$) lit. $[\alpha]$ + 265 [14], CD curve (MeOH) $[\theta]_{245}$ + 62000. IR (film) 2910, 1619, 1465, 1384, 1373, 1335, 1294, 1270, 1212, 1173, 1158, 1065, 1033, 999, 960, 920, 881 and 808 cm^{-1}, MS 204 (M^+), 189, 175, 161 (base peak, $M^+ - C_3H_7$), 147, 145, 133, 119, 105 and 91.

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