Phytochemistry, Vol. 21, No. 7, pp. 1725–1731, 1982.
Printed in Great Britain.

0031-9422/82/071725-07$03.00/0

NEW 5-METHYLCOUMARINS FROM *ETHULIA CONYZOIDES**

VISHNU S. SHUKLA†, SUBHASH C. DUTTA†, ROBINDRA N. BARUAH†, RAM P. SHARMA†, GOPALAKRISHNA THYAGARAJAN†, WERNER HERZ‡, NARENDRA KUMAR‡, KINZO WATANABE‡ and JOHN F. BLOUNT§

†Department of Organic Chemistry, Regional Research Laboratory, Jorhat-785 006, Assam, India; ‡Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.; §Research Division, Hoffmann-La Roche Inc., Nutley, NJ 07110, U.S.A.

(*Received* 21 *September* 1981)

Key Word Index—*Ethulia conyzoides*; Compositae; X-ray analysis; terpenoid 5-methylcoumarins.

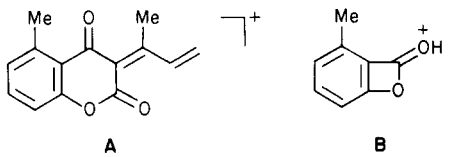
Abstract—The isolation of three new terpenoid 5-methylcoumarins in the form of diastereomeric mixtures from a north Indian collection of *Ethulia conyzoides* is reported. Stereochemistries were deduced by X-ray analysis of a pure acetate isomer obtained on acetylation of one of the diastereomeric mixtures.

INTRODUCTION

Recent work on *Ethulia conyzoides* (Vernonieae) from the USA and Egypt has resulted in the isolation of several closely related terpenoid 5-methylcoumarins exemplified by ethuliacoumarin (**1**) and cycloethuliacoumarin (**2**) [1–3]. Before we became aware of these results, our interest in biologically active lactones had prompted us to examine a north Indian collection of this species. We found no sesquiterpene lactones but now describe the isolation and structure determination of some other previously unreported terpenoid 5-methylcoumarins.

RESULTS AND DISCUSSION

The $CHCl_3$ extract of the aerial portions of *E. conyzoides* yielded two fractions which while crystalline and seemingly homogeneous on TLC were obviously mixtures after inspection of the ^{1}H-NMR spectra. The less polar material was a mixture of four diastereomers (**3a–d**) ($C_{20}H_{20}O_5$), as evidenced by typical 5-methylcoumarin frequencies [1] each of which was split or doubly split, quadrupled H-2′ and H-8′ signals near δ 6.3 and δ 6 or 5.8, a doubled H-6′ signal near δ 5.8, a complex set of superimposed H-1′ signals near δ 5.1, quadrupled sets of H-4′ signals near δ 2.2 and vinyl methyl frequencies near δ 1.95 and a doublet-up C-3′ methyl signal near δ 1.6 (see Experimental). The more polar fraction consisted of four acids (**4a, b** and **5a, b**) (see below). Mass spectra of both fractions exhibited strong peaks at *m/z* 228 and *m/z* 135 (ions **A** and **B**) [1–3] characteristic of the proposed structures.



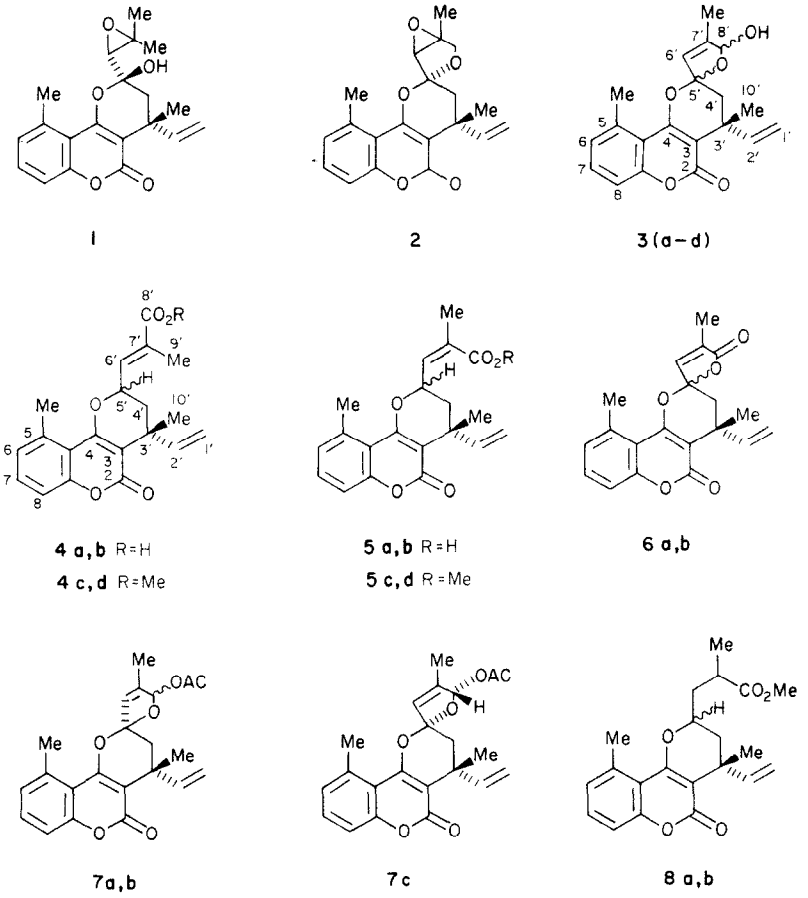
A B

The attribution of the quadrupled H-8′ signal of **3** to a set of hemiacetal protons in two pairs of epimeric hemiacetals was confirmed by oxidation (Jones' reagent) of mixture **3** to a mixture (approx. ratio 3:2) consisting of two diastereomeric γ-lactones (**6a, b**) ($C_{20}H_{18}O_5$). In comparison with **3** the ^{1}H-NMR spectrum of mixture **6** was simplified considerably thus lending itself to spin-decoupling; it also lacked the signals due to the hemiacetal protons of mixture **3** and the frequency of H-6′, now on a conjugated double bond, had shifted to lower field. The spectra of the two components **6a** and **6b** differed only in minor detail, i.e. in the chemical shifts of H-2′, H-4′, H-6′ and H-10′.

Finally, acetylation of mixture **3** and separation of the products by TLC gave a crystalline mixture of two acetates (**7a, b**) ($C_{22}H_{22}O_6$, ratio approx. 2:1) and a third pure acetate isomer (**7c**). The acetate of the 3-epimer present in least amount either escaped detection or the corresponding hemiacetal was converted to **7** under the acetylation conditions. The ^{1}H-NMR spectra of the three acetates differed from those of the four components of mixture **3** primarily in the chemical shifts of H-8′ and again differed from each other only in minor detail.

To provide a sound basis for discussing the stereochemistry of the various isomers present in **3**, single crystals of **7c** were examined by X-ray analysis. Crystal data are given in the Experimental section. Figure 1 is a stereoscopic drawing of the molecule which represents the relative configuration depicted in the plane as **7c**. The C-3′ methyl group and the side chain on C-5′ are *cis*. The pyran ring attached to C-3 and C-4 of the coumarin system is a somewhat distorted half-chair with the vinyl group on C-3′ more axially disposed than in a true half-chair. This places the vinyl group in what is essentially a 1,3-diaxial relationship to the oxygen (O-4) linking C-5′ and C-8′. Tables 1–5 list final atomic parameters and final anisotropic thermal parameters, bond lengths, bond angles and selected torsion angles.

*Dedicated to the memory of Willy Leimgruber, deceased 8 July 1981.



Chemical shifts of H-2′ and H-10′ in **7a** and **7b** are essentially indistinguishable and identical with those in components **a** and **c** of the **3a–d** mixture, while the shifts of H-8′ differ significantly (ΔδH-8′ 0.7) as would be expected from a pair of C-8′ epimers. A small difference in the shifts of the aromatic methyl group (Δδ 0.07) is also found whenever two C-8′ epimers are encountered and is easily rationalized by inspection of Fig. 1 which indicates that an α-hydroxy or α-acetoxy group would be expected to deshield the methyl group slightly. The H-2′ frequency of **7c** is significantly downfield from H-2′ of **7a, b** (Δδ H-2 = 0.15) and similar to H-2′ of **3b, d**, while ΔδH-10′ is quite small (−0.03 or −0.05). This suggests that the components of the **7a, b** mixture are C-5′ rather than C-3′ epimers of **7c**. Attempts to verify this by NOE measurements were inconclusive. ΔδH-2′ and ΔδH-10′ for two components of the **6a, b** pair (0.11 and

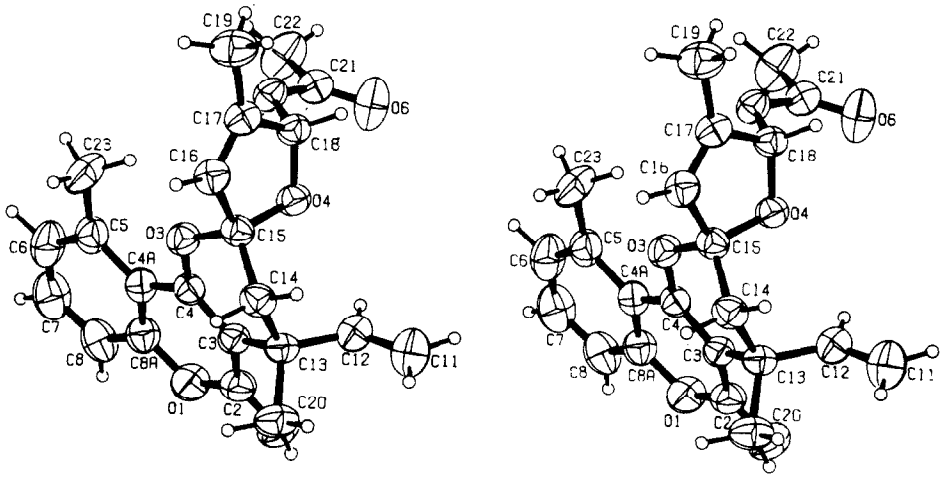


Fig. 1. Stereoscopic view of **7c**.

Table 1. Final atomic parameters for **7c** with standard deviations in parentheses

Atom	*X*	*Y*	*Z*	*B*
O(1)	0.4064(3)	0.1247(3)	0.0910(2)	*
O(2)	0.6087(3)	0.2030(3)	0.1261(2)	*
O(3)	0.1771(3)	0.4164(2)	0.1635(1)	*
O(4)	0.2994(2)	0.5940(2)	0.1342(1)	*
O(5)	0.1237(3)	0.6616(2)	0.0572(1)	*
O(6)	0.3064(3)	0.7249(3)	−0.0085(2)	*
C(2)	0.4811(4)	0.2159(3)	0.1243(2)	*
C(3)	0.4010(4)	0.3161(3)	0.1549(2)	*
C(4)	0.2595(4)	0.3236(3)	0.1408(2)	*
C(4)A	0.1821(4)	0.2294(4)	0.1027(2)	*
C(5)	0.0328(4)	0.2302(4)	0.0874(2)	*
C(6)	−0.0238(5)	0.1266(5)	0.0566(2)	*
C(7)	0.0578(6)	0.0273(5)	0.0374(2)	*
C(8)	0.2027(5)	0.0283(4)	0.0490(2)	*
C(8)A	0.2613(4)	0.1299(4)	0.0815(2)	*
C(11)	0.6887(6)	0.5459(5)	0.1778(3)	*
C(12)	0.5792(4)	0.4860(4)	0.1573(2)	*
C(13)	0.4795(4)	0.4079(3)	0.2011(2)	*
C(14)	0.3669(4)	0.4859(3)	0.2382(2)	*
C(15)	0.2466(4)	0.5239(3)	0.1911(2)	*
C(16)	0.1304(4)	0.6011(3)	0.2217(2)	*
C(17)	0.1019(4)	0.6934(3)	0.1802(2)	*
C(18)	0.2031(4)	0.6896(3)	0.1192(2)	*
C(19)	−0.0071(5)	0.7912(4)	0.1847(2)	*
C(20)	0.5634(4)	0.3404(4)	0.2589(2)	*
C(21)	0.1908(4)	0.6815(4)	−0.0044(2)	*
C(22)	0.1015(5)	0.6412(5)	−0.0645(2)	*
C(23)	−0.0652(4)	0.3345(5)	0.1030(3)	*
H(6)	−0.128	0.123	0.048	6.0
H(7)	0.013	−0.045	0.015	7.0
H(8)	0.262	−0.043	0.035	6.0
H(11)A	0.744	0.594	0.144	10.0
H(11)B	0.715	0.544	0.229	10.0
H(12)	0.558	0.493	0.106	5.0
H(14)A	0.327	0.438	0.278	4.0
H(14)B	0.414	0.561	0.257	4.0
H(16)	0.082	0.584	0.267	4.0
H(18)	0.255	0.769	0.114	3.5
H(19)A	0.034	0.862	0.211	5.5
H(19)B	−0.093	0.760	0.210	5.5
H(19)C	−0.034	0.818	0.137	5.5
H(20)A	0.637	0.287	0.237	6.0
H(20)B	0.497	0.290	0.288	6.0
H(20)C	0.611	0.402	0.290	6.0
H(22)A	0.123	0.553	−0.075	9.0
H(22)B	0.123	0.692	−0.107	9.0
H(22)C	−0.001	0.649	−0.052	9.0
H(23)A	−0.106	0.324	0.151	7.0
H(23)B	−0.144	0.335	0.068	7.0
H(23)C	−0.012	0.412	0.100	7.0

*Anisotropic thermal parameters are given in Table 2.

Table 2. Final anisotropic thermal parameters for **7c** with standard deviations in parentheses

Atom	B11 × 10⁴	B22 × 10⁴	B33 × 10⁵	B12 × 10⁴	B13 × 10⁴	B23 × 10⁴
O(1)	137(4)	79(3)	428(10)	9(3)	−1(2)	−14(1)
O(2)	115(4)	115(3)	480(12)	29(3)	−1(2)	−10(2)
O(3)	96(3)	74(2)	300(7)	3(3)	1(1)	−3(1)
O(4)	90(3)	84(2)	241(7)	13(3)	3(1)	5(1)
O(5)	89(3)	96(3)	228(7)	0(2)	−4(1)	−1(1)
O(6)	150(4)	175(4)	323(9)	−64(4)	8(2)	−10(2)
C(2)	119(6)	78(4)	305(13)	15(4)	0(2)	1(2)
C(3)	94(5)	77(3)	218(10)	2(4)	−1(2)	4(2)
C(4)	100(5)	64(3)	226(10)	2(4)	5(2)	2(2)
C(4)A	107(5)	83(4)	240(11)	−13(4)	0(2)	1(2)
C(5)	120(6)	111(5)	282(13)	−18(5)	1(2)	8(2)
C(6)	140(6)	143(6)	367(15)	−53(6)	−9(3)	−8(3)
C(7)	206(8)	121(6)	374(15)	−56(6)	−6(3)	−11(3)
C(8)	196(8)	79(4)	382(15)	−27(5)	3(3)	−8(2)
C(8)A	126(6)	82(4)	299(13)	−6(4)	2(2)	−3(2)
C(11)	160(7)	146(6)	672(24)	−44(6)	−10(4)	7(3)
C(12)	110(5)	101(4)	339(13)	−7(4)	−4(2)	1(2)
C(13)	100(5)	72(3)	243(10)	8(4)	−6(2)	1(2)
C(14)	122(5)	82(3)	218(10)	−1(4)	−7(2)	3(2)
C(15)	102(4)	71(3)	211(10)	4(4)	1(2)	2(2)
C(16)	108(5)	84(4)	230(10)	8(4)	7(2)	−4(2)
C(17)	103(5)	77(4)	247(11)	8(4)	0(2)	−8(2)
C(18)	95(5)	70(3)	234(11)	4(3)	−6(2)	−1(2)
C(19)	157(6)	111(5)	358(14)	44(5)	7(3)	−7(2)
C(20)	143(6)	106(4)	312(13)	20(5)	−22(3)	0(2)
C(21)	109(5)	87(4)	260(12)	6(4)	−1(2)	−4(2)
C(22)	152(6)	203(7)	272(13)	−12(6)	−12(3)	−19(3)
C(23)	86(5)	154(6)	522(18)	0(5)	−13(3)	(3)

The anisotropic temperature factor has the form

$$\exp(-(h^2B11 + k^2B22 + l^2B33 + 2hkB12 + 2hlB13 + 2klB23)).$$

Table 3. Bond lengths (A) in **7c** with standard deviations in parentheses

O(1) –C(2)	1.377(5)	C(5) –C(6)	1.385(7)
O(1) –C(8)A	1.378(5)	C(5) –C(23)	1.498(6)
O(2) –C(2)	1.210(5)	C(6) –C(7)	1.380(8)
O(3) –C(4)	1.349(4)	C(7) –C(8)	1.381(8)
O(3) –C(15)	1.446(4)	C(8) –C(8)A	1.387(6)
O(4) –C(15)	1.417(4)	C(11)–C(12)	1.283(7)
O(4) –C(18)	1.414(4)	C(12)–C(13)	1.519(5)
O(5) –C(18)	1.430(4)	C(13)–C(14)	1.534(5)
O(5) –C(21)	1.351(4)	C(13)–C(20)	1.542(5)
O(6) –C(21)	1.189(5)	C(14)–C(15)	1.503(5)
C(2) –C(3)	1.452(5)	C(15)–C(16)	1.500(5)
C(3) –C(4)	1.360(5)	C(16)–C(17)	1.311(5)
C(3) –C(13)	1.528(5)	C(17)–C(18)	1.503(5)
C(4) –C(4)A	1.457(5)	C(17)–C(19)	1.485(6)
C(4)A–C(5)	1.434(6)	C(21)–C(22)	1.487(6)
C(4)A–C(8)A	1.381(6)		

−0.07) and for the two C-8′ epimeric pairs in mixture **3** (0.10 and −0.08) are of the same order of magnitude, but the differences between Δδ H-2′ and Δδ H-10′ in these instances are not as pronounced as in the case of **7a, b** and **7c**.

We now discuss the more polar fraction of the *E. conyzoides* extract which on the basis of spectral analysis consisted of two pairs of *E* and *Z* isomers ($C_{20}H_{20}O_5$) of formulas **4a, b** and **5a, b**. The presence of a carboxyl group and a conjugated double bond was evidenced by the IR spectrum and conversion to a mixture of methyl esters **4c, d** and **5c, d**. In the NMR spectra, the signals of H-1′, H-2′, H-4′, H-9′, H-10′ and the 5-methyl coumarin moiety were quite similar to those found in the NMR spectra of **3**, **6** and **7** many appearing in duplicate and some exhibiting additional splitting indicative of the presence of up to four isomers. Additional signals whose relative intensity depended on the nature of the sample were two doublets near δ 6.9 and 6.2 (total intensity one proton) coupled to two broadened triplets near δ 5.6 or 5.1 (total intensity one proton). This observation could be explained by invoking the presence of *E, Z* mixture **4a, b** and **5a, b**, with H-6′ of the two *E* isomers responsible for the lower field doublet near δ 6.9 and H-6′ of the two *Z* isomers responsible for the higher field doublet near δ 6.2, analogous to the chemical shift difference exhibited by H-3 in tiglic and angelic acid. The chemical shift difference of H-5′ responsible for the broadened triplets near δ 5.6 and 5.1 could be rationalized by assuming stereoisomerism at either C-3′ or C-5′, although in

Table 4. Bond angles (°) in **7c** with standard deviations in parentheses

C(2)	-O(1)	-C(8)A	122.4(3)
C(4)	-O(3)	-C(15)	118.0(3)
C(15)	-O(4)	-C(18)	109.4(3)
C(18)	-O(5)	-C(21)	116.0(3)
O(1)	-C(2)	-O(2)	115.7(3)
O(1)	-C(2)	-C(3)	117.9(3)
O(2)	-C(2)	-C(3)	126.3(4)
C(2)	-C(3)	-C(4)	118.3(3)
C(2)	-C(3)	-C(13)	118.6(3)
C(4)	-C(3)	-C(13)	123.1(3)
O(3)	-C(4)	-C(3)	123.1(3)
O(3)	-C(4)	-C(4)A	114.0(3)
C(3)	-C(4)	-C(4)A	122.9(3)
C(4)	-C(4)A	-C(5)	125.9(4)
C(4)	-C(4)A	-C(8)A	115.8(3)
C(5)	-C(4)A	-C(8)A	118.3(4)
C(4)A	-C(5)	-C(6)	117.2(4)
C(4)A	-C(5)	-C(23)	124.6(4)
C(6)	-C(5)	-C(23)	118.2(4)
C(5)	-C(6)	-C(7)	123.0(4)
C(6)	-C(7)	-C(8)	120.0(5)
C(7)	-C(8)	-C(8)A	118.0(4)
O(1)	-C(8)A	-C(4)A	121.9(3)
O(1)	-C(8)A	-C(8)	114.8(4)
C(4)A	-C(8)A	-C(8)	123.3(4)
C(11)	-C(12)	-C(13)	128.0(4)
C(3)	-C(13)	-C(12)	110.6(3)
C(3)	-C(13)	-C(14)	107.4(3)
C(3)	-C(13)	-C(20)	110.1(3)
C(12)	-C(13)	-C(14)	111.4(3)
C(12)	-C(13)	-C(20)	110.3(3)
C(14)	-C(13)	-C(20)	107.0(3)
C(13)	-C(14)	-C(15)	113.5(3)
O(3)	-C(15)	-O(4)	108.8(2)
O(3)	-C(15)	-C(14)	109.4(3)
O(3)	-C(15)	-C(16)	105.7(3)
O(4)	-C(15)	-C(14)	110.1(3)
O(4)	-C(15)	-C(16)	104.3(3)
C(14)	-C(15)	-C(16)	118.2(3)
C(15)	-C(16)	-C(17)	110.5(3)
C(16)	-C(17)	-C(18)	108.3(3)
C(16)	-C(17)	-C(19)	131.6(4)
C(18)	-C(17)	-C(19)	120.1(3)
O(4)	-C(18)	-O(5)	110.0(3)
O(4)	-C(18)	-C(17)	105.7(3)
O(5)	-C(18)	-C(17)	108.3(3)
O(5)	-C(21)	-O(6)	123.2(3)
O(5)	-C(21)	-C(22)	110.9(3)
O(6)	-C(21)	-C(22)	125.9(4)

view of the data for **3**, **5**, **7** and earlier results [1–3] the latter seemed more plausible. Catalytic hydrogenation of the methyl ester mixture afforded a mixture of equal amounts of two (by NMR criteria) tetrahydro derivatives **8** whose ^{1}NMR spectrum exhibited the expected chemical changes, i.e., disappearance of the vinylic H-1′, H-2′ and H-6′ frequencies, appearance of a methyl triplet (H-1′) and a methyl doublet (H-9′) and upfield shift of the H-5′ signal, now coupled to four protons, to δ 4.19.

Table 5. Torsion angles (°) in **7c** with standard deviations in parentheses

O(1)	-C(2)	-C(3)	-C(4)	-8.9(5)
C(2)	-C(3)	-C(4)	-C(4)A	6.5(5)
C(3)	-C(4)	-C(4)A	-C(8)A	0.4(5)
C(4)	-C(4)A	-C(8)A	-O(1)	-5.0(5)
C(4)A	-C(8)A	-O(1)	-C(2)	2.4(6)
C(8)A	-O(1)	-C(2)	-C(3)	4.7(5)
C(13)	-C(3)	-C(4)	-O(3)	3.6(5)
C(3)	-C(4)	-O(3)	-C(15)	13.1(5)
C(4)	-O(3)	-C(15)	-C(14)	-43.4(4)
O(3)	-C(15)	-C(14)	-C(13)	59.4(4)
C(15)	-C(14)	-C(13)	-C(3)	-42.5(4)
C(14)	-C(13)	-C(3)	-C(4)	11.7(5)
O(4)	-C(15)	-C(16)	-C(17)	10.3(4)
C(15)	-C(16)	-C(17)	-C(18)	-2.9(4)
C(16)	-C(17)	-C(18)	-O(4)	-5.7(4)
C(17)	-C(18)	-O(4)	-C(15)	12.4(3)
C(18)	-O(4)	-C(15)	-C(16)	-13.8(3)

EXPERIMENTAL

Extraction of E. conyzoides. Above ground parts of *E. conyzoides* L. (1 kg), collected in the Sibsagar District of Assam, India (voucher on deposit in the Herbarium of RRL, Jorhat) were extracted with $CHCl_3$ in a Soxhlet apparatus until the extract was colourless. After removal of $CHCl_3$ at red. pres. the residue was dissolved in 350 ml MeOH containing 10% H_2O, allowed to stand overnight and filtered. The filtrate was washed with petrol (bp 60–80°, 6 × 300 ml), the MeOH portion was concd at red. pres. and thoroughly extracted with $CHCl_3$. The washed and dried residue was evaporated at red. pres. and the residue (17 g) chromatographed over 300 g Si gel (60–120 mesh, BDH, India), 200 ml fractions being collected in the following order: Fr. 1–5 petrol–$CHCl_3$ (9:1), 6–10 petrol–$CHCl_3$ (2:1), 11–15 petrol–$CHCl_3$ (1:1), 16–20 $CHCl_3$, 21–25 $CHCl_3$–MeOH (49:1), 26–35 $CHCl_3$–MeOH (19:1), 36–50 $CHCl_3$–MeOH (9:1) and 51–55 $CHCl_3$–MeOH (4:1). Fr. 35–37 (6.8 g) which showed two major spots on TLC were combined and the substances responsible for the spots were separated by prep. TLC (C_6H_6–EtOAc, 4:1).

The less polar material **3** (1.5 g) was recrystallized from MeOH, mp 193–194°, but remained a mixture of isomers and decomposed slowly on standing; ^{1}H-NMR signals (270 MHz, $CDCl_3$, isomers are designated alphabetically in order of decreasing quantity): δ 7.30 (*t*, J = 8 Hz, slightly split, H-7 of **abcd**), 7.13 (*d* (*br*), J = 8 Hz, H-8 of **abcd**), 6.98 (*d* (*br*), J = 8 Hz, H-6 of **abcd**), 6.32, 6.31, 6.12 and 6.11 (all *dd*, J = 17.5, 10 Hz, H-2′ of **dbac**), 6.02 (*br*), 5.99 (*br*), 5.77 (*br*), 7.75 (*br*, H-8′ of **cdab**), 5.85 (*br*) and 5.79 (*br*, H-6′ of major and minor pair), 5.09 (*c*, H-1′ a, b of **abcd**), 2.67, 2.66, 2.60, 2.59 (arom. Me of **abcd**), 2.34 (*d*) and 2.15 (*d*, J = 14 Hz, H-4′ of **d**), 2.33 (*d*) and 2.14 (*d*, J = 14 Hz, H-4′ a, b of **c**), 2.28 (*d*) and 2.03 (*d*, J = 14 Hz, H-4′ a, b of **a** or **b**), 2.16 (H-4′ ab of **b** or **a**), 1.96, 1.95, 1.94 and 1.93 (H-9′ of **c** or **d**, **a**, **b**, **d** or **c**), 1.69 and 1.61 (H-10′ of major and minor pair). Mass spectra are reported for the acetates.

The more polar fraction (**4a**, **b** and **5a**, **b**) yield 1.87 g, had mp 197°; the ^{1}H-NMR spectrum (see below) indicated that it was a mixture. An attempt at acetylation resulted in recovery of starting material; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600–2700 (*br*) and 1720 (*br*, carboxyl), 1660 (*d*(*br*)), 1600, 1580, 1110 and

1090; ^{1}H-NMR (270 MHz, $CDCl_3$); δ 7.35 (*t*, $J = 8$ Hz, lines split, H-7 of two or more isomers), 7.15 (*d* (*br*), $J = 8$ Hz, H-8), 7.03 (*d* (*br* $J = 8$ Hz, H-6) superimposed on *d* (H-6′ of *E*-isomers) coupled to signal at 5.09 (*m*, H-5′), 6.35 (*d* (*br*), $J = 7.5$ Hz, weak, H-6′ of *Z*-isomers) coupled to 5.65 (*t* (*br*), $J = 7.5$ Hz, weak, H-5′), 6.18 (*dd*, $J = 18$, 10 Hz, each line triply split, H-2′), 5.16 (*d*, $J = 18$ Hz) and 5.15 (*d*, $J = 10$ Hz, H-1′ a, b) 2.69 and 2.67 (5-Me), 2.08 and 2.00 (H-9′), the latter superimposed on H-4′a, 1.84 (*dd*, $J = 14$, 1.5 Hz, H-4′b), 1.68 (H-10′); ^{1}H-NMR (270 MHz, C_6D_6): δ 7*c* (H-6, H-7 and H-8), 6.76 (*d*) and 6.74 (*d*, $J = 7.5$ Hz, H-6′ of two *E*-isomers), 4.43 (*m*, H-5′ of *E*-isomers), 6.30 (*dd*, $J = 18$, 10 Hz, each line split, H-2′) 6 (*d*) and 5.3 (*t* (*br*) weak, H-6′ and H-5′ of *Z*-isomers), 5.10 (*d*, $J = 10$ Hz) and 5.07 (*d*, $J = 18$ Hz, H-1′ a, b), 2.48 and 2.43 (5-Me), 1.79 (center of AB system, H-4′ a, b of one isomer pair), 1.60 (*c*) and 1.30 (*d*, $J = 14$, H-4′, a, b of the other isomer pair), 1.64 and 1.47 (H-9′ and H-10′). [(Calc. for $C_{20}H_{20}O_5$: MW, 340.1309. Found: MW(MS), 340.1318 (9.5%).] Other significant peaks in the high resolution MS were at *m/z* rel. int. 325 $[C_{19}H_{17}O_5]^+$ (16.1), 322 $[C_{20}H_{18}O_4]^+$ (4.9), 307 $[C_{19}H_{15}O_4]^+$ (10.0), 2.95 $[C_{19}H_{19}O_3]^+$ (6.5), 294 $[C_{19}H_{18}O_3]^+$ (8.8), 279 $[C_{18}H_{15}O_3]^+$ (11.9), 271 $[C_{15}H_{11}O_5]^+$ (2.2), 267 $[C_{17}H_{15}O_3]^+$ (6.0), 241 $[C_{15}H_{13}O_3]^+$ (5.2), 230 $[C_{14}H_{14}O_3]^+$ (9.8), 228 $[C_{14}H_{12}O_3]^+$ (57.8, ion **A**), 227 $[C_{14}H_{11}O_3]^+$ (49.1), 226 $[C_{14}H_{10}O_3]^+$ (9.8), 215 $[C_{13}H_{11}O_3]^+$ (15.4), 214 $[C_{13}H_{10}O_3]^+$ (14.0), 213 $[C_{13}H_9O_3]^+$ (3.3), 199 $[C_{11}H_{11}O_2]^+$ (10.8), 173 $[C_{11}H_9O_2]^+$ (52.7), 145 $[C_{10}H_9O]^+$ (14.1), 135 $[C_8H_7O_2]^+$ (100, ion **B**), 106 $[C_7H_6O]^+$ (22.3), 105 $[C_7H_5O]^+$ (19.1).

Oxidation of **3**. A soln of 40 mg mixture **3** in 15 ml of acetone and 0.2 ml Jones' reagent was stirred for 20 min at 5–10°. Excess reagent was destroyed by addition of MeOH. The mixture was diluted with H_2O and extracted with $CHCl_3$. Evaporation of the washed and dried extract furnished **6a, b** (3:2 mixture) as a solid, mp 166–167° (EtOAc). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1780, 1700, 1650 and 1600; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.36 (*t*, $J = 8$ Hz, H-7 of **a**, **b**), 7.1 (*d* (*br*), $J = 8$ Hz, H-8 of **a, b**), 7.00 (*d* (*br*), $J = 8$ Hz, H-6 of **a, b**), 7.03 (*d*, $J = 1.5$ Hz, H-6′ of **a** major constituent), 6.98 (*d*, $J = 1.5$ Hz, H-6′ of **b** minor constituent), 6.25 (*dd*) and 6.16 (*dd*, $J = 18$, 10 Hz, H-2′ of **b** and **a**), 5.18 (*d*, $J = 10$ Hz, H-1′ a of **a, b**), 5.19 (*d*), 5.12 (*d*, $J = 18$ Hz, H-1′ b of **b** and **a**), 2.56 and 2.56 (5-Me of **a, b**) 2.39 (*d*) and 2.14 (*d*, $J = 14$ Hz, H-4′ of **a**), 2.27 (*br*, 2p, H-4′ of **b**), 2.04 (H-9′ of **a, b**), 1.74 and 1.67 (H-10′ of **a** and **b**); MS: significant peaks at *m/z* 338 $[M]^+$, 323 $[M - Me]^+$, 320, 309, 295, 293, 281, 279, 277, 267, 265, 241, 228 (ion **A**), 215, 213, 204, 199, 186, 135 (ion **B**, base peak), 128, 125, 123, 106, 105.

Acetylation of **3**. Acetylation of 1.5 g mixture **3** with 6 ml Ac_2O and 3 ml pyridine at room temp. overnight followed by the usual work-up gave material which showed two spots on TLC. Separation by prep. TLC (C_6H_6–EtOAc, 9:1) gave as the more polar fraction 0.7 g substance **7c**, mp 90° (MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1715, 1630, 1600, 1555, 1200, 970, 915; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.25 (*t*, $J = 8$ Hz, H-7), 7.11 (*d* (*br*), $J = 8$ Hz, H-8), 7.01 (*d* (*br*), $J = 8$ Hz, H-6), 6.68 (H-8′), 6.27 (*dd*, $J = 18$, 10 Hz, H-2′), 5.93 (*br*, H-6′), 5.16 (*d*, $J = 18$ Hz) and 5.06 (*d*, $J = 10$ Hz, H-1′ a, b), 2.65 (5-Me), 2.24 (*d*, $J = 14$ Hz) and 2.14 (*d*, $J = 14$ Hz, H-4′ a, b), 2.03 (Ac), 1.89 (*br*, H-9′), 1.61 (H-10′); ^{13}C-NMR (67.9 MHz, $CDCl_3$, probable assignments): singlets 169.61 (acetate C=O), 160.15 (C-2), 154.06, 141.35, 137.26, 114.5, 110.26, 110.26, 107.54, 35.87 (C-3′), doublets 143.93 (C-2′), 130.73 (C-7), 127.86 and 127.37 (C-6 and C-6′), 114.64 (C-8), 100.78 (C-8′), triplets 112.0 (C-1′), 44.16 (C-4′), quartets 25.69 (C-10′), 23.56 (5-Me), 20.84 (Ac), 11.59 (C-9′); UV λ_{max}^{MeOH} nm (ϵ): 221 (20400), 269sh, (11900), 291.5 (15700), 306sh (9900), 320sh (6000). [Calc. for $C_{22}H_{22}O_6$: MW, 382.1415. Found: MW(MS), 382.1393 (3.3%).] Other significant peaks in the high resolution MS were at *m/z* (rel. int.) 340 $[C_{20}H_{20}O_5]^+$ (1.2), 329 $[C_{20}H_{19}O_4]^+$ (17.9), 328 $[C_{20}H_{28}O_4]^+$ (24.2), 307 $[C_{19}H_{15}O_4]^+$ (17.2), 295 $[C_{15}H_{19}O_6]^+$ (4.9), 293 $[C_{19}H_{17}O_3]^+$ (6.5), 279 $[C_{18}H_{15}O_3]^+$ (5.8), 241 $[C_{15}H_{13}O_3]^+$ (4.6), 228 $[C_{14}H_{12}O_3]^+$ (53.1), 227 $[C_{14}H_{11}O_3]^+$ (29.5), 214 $[C_{13}H_{10}O_3]^+$ (14.6), 213 $[C_{13}H_9O_3]^+$ (14.7), 188 $[C_{12}H_{12}O_2]^+$ (41.3), 171 $[C_{12}H_{11}O]^+$ (6.3), 170 $[C_{12}H_{10}O]^+$ (7.1), 160 $[C_{11}H_{12}O]^+$ (9.5), 148 $[C_{10}H_{12}O]^+$ (8.2), 135 $[C_8H_7O_2]^+$ (48.5), 112 $[C_8H_8O_2]^+$ (100), 108 $[C_7H_8O]^+$ (29.2).

The less polar fraction (0.7 g) was a 2:1 mixture of two acetates **7a, b**, mp 78° (MeOH) whose IR and MS were essentially identical with the IR and MS of **7c**. ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.33 (*t*, $J = 8$ Hz, H-7), 7.12 (*d* (*br*), $J = 8$ Hz, H-8), 6.99 (*d* (*br*), $J = 8$ Hz, H-6), 6.81 and 6.64 (H-8′ of **b** and **a**), 6.12 (*dd*) and 6.11 (*dd*, $J = 18$, 10 Hz, H-2′ of **b** and **a**), 5.98 (*br*) and 5.95 (*br*, H-6′ of **a** and **b**), 5.11 (*m*, 2p, H-1′ a, b of **a, b**), 2.67 and 2.60 (5-Me of **a** and **b**), 2.28 (*d*, $J = 14$ Hz, each line narrowly split H-4′ a of **a, b**), 2.14 and 2.05 (Ac of **b** and **a**), 2.04 (*d*, $J = 14$ Hz, each line narrowly split, H-4′ b of **a, b**), 1.89 (H-9′), 1.66 and 1.64 (H-10′ of **a** and **b**). The off resonance ^{13}C-NMR spectrum indicated the presence of only two compounds, many of the lines found earlier in the spectrum of **7c** being doubled, viz. 169.65 (Ac C=O), 160.10 (C-2), 153.95, 144.93 and 144.67, 140.44, 137.16, 130.78, 127.86, 127.37, 114.72, 114.26, 112.72 and 112.45, 110.57, 105.10, 101.61 and 100.34, 44.03 and 43.02, 36.53 and 36.35, 25.22 and 24.69, 23.73 and 23.68, 20.93, 11.73 and 11.59.

Reactions of **4** *and* **5**. Methylation of 50 mg of the mixture **4a, b** and **5a, b** with CH_2N_2 overnight followed by the usual work-up gave 50 mg of a mixture of **4c, d** and **5c, d** as a gum. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1700, 1660, 1560, 1125 and 1000; ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.35 (*t*, $J = 8$ Hz, overlapping signals of H-7), 7.12 (*d* (*br*), $J = 8$ Hz, H-8), 7.01 (*d* (*br*), $J = 8$ Hz, each line split, H-6), 6.89 (*d*, $J = 7.5$ Hz, narrowly split, H-6′ of *E*-isomers), 6.18 (*dd*, $J = 18$, 10 Hz, each line split four ways; H-2′ of four isomers) superimposed on 6.1 (*m*, H-6′ of *Z*-isomers), 5.58 (*t* (*br*), $J = 7.5$ Hz, each line split, H-5′ of 2 isomers), 5.12 (*m*, H-1′ a, b superimposed on 5.05 (*m*, H-5′ of 2 isomers), 3.80, 3.77 (OMe), 2.66 and 2.64 (5-Me), 2.03 and 1.97 (H-9′), 1.9 (*c*, H-4′), 1.67 and 1.63 (H-10′); MS *m/z*: 354 $[M]^+$, 339 $[M - Me]^+$, 322 $[M - MeOH]^+$, 307, 298, 294, 279, 267, 254, 241, 228 (ion **A**), 227, 215, 214, 213, 199, 187, 185, 173, 144, 135 (base peak, ion **B**), 106, 105. Catalytic hydrogenation of 30 mg of **4c, d 5c, d** mixture in 25 ml EtOAc with 0.1 g Pd/C for 6 hr at 3 atm, filtration, evaporation and prep. TLC of the residue (C_6H_6–EtOAc, 9:1) afforded a mixture of esters (20 mg) which exhibited ^{1}H-NMR signals (270 MHz, $CDCl_3$) at δ 7.32 (*t*, $J = 8$ Hz, H-7), 7.13 (*d* (*br*), $J = 8$ Hz, H-6), 7.01 (*d* (*br*), $J = 8$ Hz, H-8), 4.19 (*c*, H-5′), 3.72 and 3.70 (OMe of minor and major component), 2.86 (*c*, H-7′ of all components), 2.69 and 2.68 (5-Me of minor and major component), 2.30 (*c*, H-2′?), 1.82 (*c*) and 1.64 (*c*, H-4′ and H-6′), 1.30 (*d*) and 1.29 (*d*, $J = 7$ Hz, H-9′ of major and minor components), 0.73 (*t*, $J = 7$ Hz, H-1′).

X-Ray analysis of **7c**. Single crystals of **7c** suitable for analysis were obtained by slow crystallization from MeOH. They were orthorhombic, space group $P2_12_12$, with $a = 9.407(3)$, $b = 10.955(3)$, $c = 19.045(5)$ Å, and $d_{calc} = 1.294$ cm^{-3} for $Z = 4$ ($C_{22}H_{22}O_6$, $M = 382.41$). The intensity data were measured on a Hilger–Watts diffractometer (Ni-filtered Cu $K\alpha$ radiation, θ–2θ scans, pulse-height discrimination). The size of the crystal used for data collection was approximately $0.25 \times 0.35 \times 0.5$ mm. A total of 1533 indepen-

dent reflections were measured for $\theta < 57°$, of which 1451 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure [4] and was refined by full-matrix least squares. Three reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement, anisotropic thermal parameters were used for the non-hydrogen atoms and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations but their parameters were not refined. The final discrepancy indices are $R = 0.042$ and $wR = 0.050$ for the remaining 1448 observed reflections. The final difference map has no peaks greater than ± 0.2 eÅ^{-3}.

Acknowledgement—Work at the Florida State University was supported in part by a grant from the U.S. Public Health Service (CA-13121) through the National Cancer Institute.

REFERENCES

1. Bohlmann, F. and Zdero, C. (1977) *Phytochemistry* **16**, 1092.
2. Baalba, S. I., Halim, A. F., Halaweish, F. T. and Bohlmann, F. (1980) *Phytochemistry* **19**, 1519.
3. Bohlmann, F., Baalba, S. I., Halim, A. F. and Halaweish, F. T. (1981) *Phytochemistry* **20**, 177.
4. Germain, G., Main, P. and Woolfson, M. M. (1971) *Acta Crystallogr.* **A27**, 368.