SESQUITERPENIC LACTONES OF SOME SPECIES OF GENUS *Vernonia* SCHREB.*

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Four species of the genus *Vernonia*, endemic in Cuba, have been investigated on the presence of sesquiterpenic lactones. In addition to two derivatives of lupeol, scopoletin, genkwanin and velutin, total 15 sesquiterpenic lactones have been isolated, four of which are described for the first time.

The genus *Vernonia* SCHREB. (family *Asteraceae*, tribe *Vernonieae*) comprises about 1 000 species of which 45 grow in Cuba, all but one being Cuban endemites[1]. Because the content of sesquiterpenic lactones in these Cuban *Vernonia* species has not been studied so far, we decided ten years ago to study at least several endemic Cuban species[2], accessible to us.

As the first we studied aerial parts of species *Vernonia angusticeps* EKM. From the chloroform extract we isolated lupeol acetate (*I*), coumarin derivative scopoletin (*II*) and three sesquiterpenic lactones: 3-oxograndolide (*III*), 3β-hydroxygrandolide (*IV*) and reynosin (*V*). The lactone *III* was isolated some time ago from the aerial part of species *Arctotis grandis* THUNB. (family *Asteraceae*, tribe *Arctotae*) and its structure as well as absolute configuration was determined[3,4]. Later on, lactone *III* was described as a constituent of species *Liabum floribundum* LESS. (*Asteraceae*, *Liabae*)[5]. Reynosin (*V*) was obtained from species *Ambrosia confertiflora* DC (*Asteraceae*, *Heliantheae*)[6], and probably also from species *Chrysanthemum parthenium* BERNH. (*Asteraceae*, *Anthemideae*)[7] and *Tanacetum vulgare* L. (*Asteraceae*, *Anthemideae*)[8]. Its structure and absolute configuration are also known[5 – 7].

Further we studied aerial parts of species *Vernonia acunnae* ALAIN. From the light petroleum and chloroform extracts we isolated in relatively high yield the known lupeol palmitate (*VI*) and five sesquiterpenic lactones. One of them, glaucolide A (*VII*), is

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relatively common in species of *Vernonia* genus from the American continent[9] and its structure has been confirmed by X-ray diffraction analysis[10]. The other lactones were piptocarphin A (*VIII*), isolated previously from species *Piptocarpha chontalensis* PALL. (*Asteraceae*, *Vernonia*e), whose structure has been established[11], and tulipinolide (*IX*), obtained more than twenty years ago from species *Liliodendron tulipifera* L. (family *Magnoliaceae*), also of known structure[12]. Later on, lactone *IX* was obtained also from *Ambrosia camphorata* (GREENE) PAYNE (*Asteraceae*, *Heliantheae*)[13]. The two further isolated lactones were guaianolides of closely similar structure; shortly before our study, one of them, 1α*H*,5α*H*,6β*H*,7α*H*,8α-hydroxyguai-4(15),10(14),11(13)-trien-6,12-olide (*X*) was described as the native compound of species *Centaurea canariensis* WILLD. (*Asteraceae*, *Cynareae*) and its structure (but not absolute configuration) was established[14]. The second lactone, 1α*H*,5α*H*,6β*H*,7α*H*,8α-acetoxyguai-4(15),10(14),11(13)-trien-6,12-olide (*XI*) was obtained from species *Gochnatia smithii* ROBINSON et GREEN (*Asteraceae*, *Mutisieae*)[15] shortly after our isolation[2].

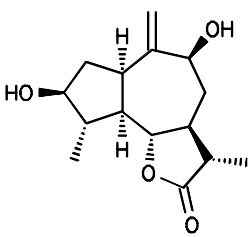
The aerial part of species *Vernonia menthaefolia* (POEPP. et SPRENG.) LESS. afforded, beside the already known flavonoids velutin (*XII*) and genkwanin (*XIII*), three undescribed sesquiterpenic lactones: 8α-(2′-methyl)acryloyloxy-13-methoxyvernojalcanolide – [1β,4α-dihydroxy-5β,10α-diacetoxy-8α-(2′-methyl)acryloyloxy-13-methoxycadin-7(11)-en-6,12-olide] (*XIV*), 8α-(2′-methyl)acryloyloxy-13-ethoxyvernojalcanolide – [1β,4α-dihydroxy-5β,10α-diacetoxy-8α-(2′-methyl)acryloyloxy-13-ethoxycadin-7(11)-en-6,12-olide] (*XV*) and 1α,10α-dihydroxy-8α-(2′-methyl)acryloyloxy-13-methoxyhirsutinolide (*XVI*). The lactone *XVI* was then isolated as the acetate from species *Vernonia jalcana* CUATR.[16].

Finally, we studied aerial parts of *Vernonia moaensis* ALAIN from which, in addition to lupeol acetate (*I*) and lupeol palmitate (*VI*), we isolated the relatively widespread sesquiterpenic lactone costunolide (*XVII*)[9] and the long time ago described dihydrodehydrocostuslactone (*XVIII*)[17]. Further we isolated 3β-hydroxy-4,15-dehydrograndolide (*XIX*) and 1α,10α-dihydroxy-8α-acetoxy-13-ethoxyhirsutinolide (*XX*). Last year, the lactone *XIX* was described as constituent of species *Venidium fastuosum* STAP. (*Asteraceae*, *Arctotae*)[18].

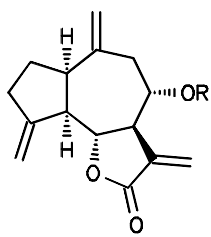
RESULTS AND DISCUSSION

As already mentioned, from species *Vernonia angusticeps* we obtained, in addition to other compounds, also 3β-hydroxygrandolide (*IV*), m.p. 162 – 165 °C, $[\alpha]_D$ +6.5°, composition $C_{15}H_{22}O_4$. Its infrared spectrum proved the presence of a hydroxy group (3 610 and 3 490 cm^{-1}), a γ-lactone (1 768 cm^{-1}) and a double bond (1 643 cm^{-1}). Its mass spectrum exhibited molecular peak 266 and characteristic fragments 248 (M – 18) and 230 (248 – 18). CD spectrum had a maximum at 216 nm (Δε +1.1). ^{1}H NMR spectrum in $CDCl_3$ proved a *trans*-annelated C-6-lactone (H-6: 3.87 t, $J(6,5) \approx J(6,7)$ = 9.8 Hz), two secondary hydroxyl groups in positions 3 and 9 (H-3: 3.73 dt; H-9: 4.07

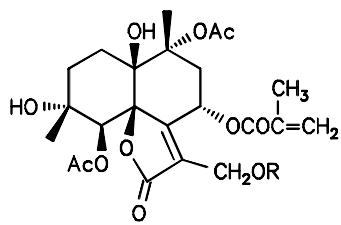
bdd), an exomethylene group in position 10 (H-14 and H-14′: 5.49 b and 5.15 d) and secondary methyl groups in positions 4 (1.20 d) and 11 (H-13: 1.24 d). The configuration of substituents in positions 6, 9 and 11, as well as *cis*-annelation at atoms C(1) and C(5), was reliably derived from the coupling constants. The determination of configuration at carbon atoms C(3) and C(4) of the five-membered ring was complicated by overlap of signals due to H-2, H-4, H-5 and H-7. Change in the solvent (use of 1 : 1 mixture of $CDCl_3$ and C_6H_6) led to partial separation of the signals and only in situ TAI-acylation, performed in this solvent mixture, resulted in such distribution of signals from which we were able to obtain the values of all the coupling constants and to use selective difference NOE. On saturation of signals of H-15 methyl protons, we observed 3% NOE of H-4, 3.5% NOE of H-3 and 2% NOE of H-5α. From this it follows that the methyl group (H-15) is *cis*-oriented relatively to the protons in posi-



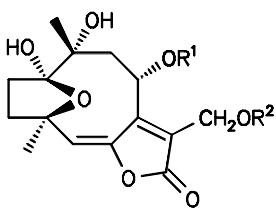
IV



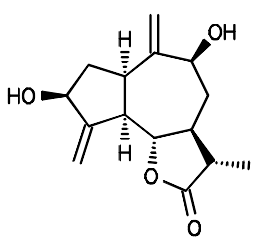
X, R = H
XI, R = $COCH_3$



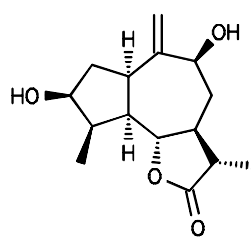
XIV, R = CH_3
XV, R = CH_2CH_3



XVI, R^1 = $COC(CH_3)=CH_2$; R^2 = CH_3
XX, R^1 = $COCH_3$; R^2 = CH_2CH_3



XIX



XXI

tions 3 and 5 and, consequently, the configuration of the substituents on the five-membered ring is 3β-OH and 4α-CH_3. The absolute configuration at C(11) in 3β-hydroxygrandolide (*IV*) is (*S*) as derived from the positive CD maximum at 216 nm using sector rule[19,20]. Thus, formula *IV* depicts also the absolute configuration of this lactone. The lactone *IV* was prepared by reduction of 3-oxograndolide (*III*) with sodium borohydride.

A detailed analysis of ^{1}H NMR spectra of the lactone *IV* from *V. angusticeps* and the lactone from *Arctotis grandis*, originally supposed to be identical with lactone *IV* (ref.[4]), showed that the compounds are different. Our new ^{1}H NMR data (Table I) show values almost identical with those found for lactone *IV*, except for the protons of the five-membered ring. As in the case of lactone *IV*, the degeneration of signals of protons in position 2 (at δ 2.06) in $CDCl_3$ was not suppressed by use of a $CDCl_3$–C_6H_6 mixture (1 : 1) (both H-2 and H-5 are again at $\delta \approx 1.85$) and good resolution was again obtained only upon TAI-acylation in the said solvent mixture. In a selective difference NOE experiment, saturation of the methyl signal (H-15) resulted in 4.5% NOE for the H-4 and 4% NOE for the H-6β protons whereas no NOE was observed for the proton H-3. Accordingly, in this lactone the substituents on the five-membered ring have configuration 3β-OH and 4β-CH_3 and the compound is 3β-hydroxy-4-epigrandolide, described by formula *XXI*. The lactone is obviously identical with the lactone described in ref.[21], originally characterized by formula *IV* which was later revised[5] in favour of the structure *XXI*.

From species *Vernonia acunnae* we as the first isolated and described[2] the sesquiterpenic lactone *XI*, m.p. 112 – 114 °C, $[\alpha]_D$ +78.8°, composition $C_{17}H_{20}O_4$. Its IR spectrum showed the presence of a γ-lactone (1 763 cm^{-1}), an acetate (1 739 and 1 245 cm^{-1}) and a double bond (1 643 and 1 657 cm^{-1}). In the mass spectrum there was molecular peak M^+ 288 and characteristic fragments 246 (M – CH_2=CO) and 228 (M – 60). CD maximum was located at 220 nm (Δε –3.8). ^{1}H NMR spectrum (Table II) proved the presence of an acetate in position 8α (OAc: 2.15 s; H-8: 4.98 dt, *J*(8,7) = 10.2, *J*(8,9) = 5.1 and 5.4 Hz), an exomethylene γ-lactone, *trans*-annelated in positions 6 and 7 (H-13: 6.25 d, *J*(13,7) = 3.5 Hz; H-13′: 5.66 d, *J*(13′,7) = 3.0 Hz; H-6: 4.02 dd, *J*(6,5) = 10.3, *J*(6,7) = 8.9 Hz), and two other exomethylene groups (signals at δ 4.94, 5.05, 5.10 and 5.30). Detailed analysis of our NMR data (which are in good accord with the published[15] incomplete 80 MHz data) led to the structure *XI*.

The structure of the further isolated lactone, *X*, was suggested on the basis of comparison of its ^{1}H NMR spectrum with that of compound *XI*. The absence of acetate group, accompanied by an upfield shift of the H-8 signal (δ 3.93), retention of signals of all other protons in the molecule and proof of a hydroxy group by TAI-acylation, have shown that compound *X* is the deacetyl derivative of lactone *XI*. This was confirmed by hydrolysis of compound *XI* which afforded lactone *X*. The physical constants as well as ^{1}H NMR data for *X* (Table II) agree well with the published ones[14]. The

TABLE I

^{1}H NMR parameters of guaianolide derivatives *IV* and *XXI*, coupling constants in parentheses, TAI-induced acylation shifts (in the mixture of $CDCl_3$ + C_6D_6) in square brackets

Proton	Compound *IV*			Compound *XXI*		
	$CDCl_3$	$CDCl_3$ + C_6D_6 (1:1)	+ TAI	$CDCl_3$	$CDCl_3$ + C_6D_6 (1:1)	+ TAI
H-1	2.77 bq (8.9 (3 ×))	2.38 bq (9.9; 9.3; 7.5)	2.64 bq [0.26] (10.1; 8.8; 7.1)	2.70 bq (11.4; 10; 10; 1.5; 1.1)	2.33 bq (~10.0 (3 ×))	2.48 bq [0.16] (11.2; 10.7; 8.3; 1.3)
H-2α	~2.23	1.99 dt (12.8; 7.5; 6.8)	2.33 dt [0.34] (13.2; 7.1; 6.8)	~2.06	~1.88	2.14 ddd [0.26] (12.8; 8.3; 7.0)
H-2β	~1.81	1.60 dt (12.8; 9.3; 9.0)	1.74 dt [0.14] (13.2; 8.9; 8.8)	~2.06	~1.83	2.08 dt [0.25] (12.8; 10.7 (2 ×))
H-3	3.73 dt (9.0 (2 ×); 6.7)	3.43 bq (9.0; 8.9; 6.8)	4.69 dt [1.26] (8.9; 8.8; 6.8)	4.26 bq (~7.5 (2 ×); 6.2)	3.95 bq (~7.0 (3 ×))	5.01 ddd [1.06] (10.7; 7.0; 6.2)
H-4	~1.81	1.64 m (9.4; 8.9; 6.6 (3 ×))	2.10 tq [0.46] (8.8; 8.6; 6.6 (3 ×))	2.36 tq (6.2 (2 ×); 7.2 (3 ×))	2.18 m (~7.0; 6.4; 7.3 (3 ×))	2.55 m [0.37] (6.4; 6.2; 7.0 (3 ×))
H-5	~1.81	1.46 q (10.3; 9.9; 9.4)	1.54 q [0.08] (10.4; 10.1; 8.6)	2.19 dt (11.4; 11.2; 6.2)	1.85 dt (11.2; 10; 6.4)	1.85 dt [0.00] (11.2 (2 ×); 6.4)
H-6	3.87 t (9.9; 9.8)	3.48 t (10.3; 9.7)	3.51 t [0.03] (10.4; 9.7)	4.06 dd (11.2; 9.7)	3.76 dd (11.2; 9.6)	3.78 dd [0.02] (11.2; 9.6)
H-7	~1.81	1.43 m (12.2; 12.0; 9.7; 3.7)	1.48 m [0.05] (12.2; 12.0; 9.7; 3.6)	1.82 ddt (12.4; 12.0; 9.7; 3.0)	1.44 ddt (12.4; 12.0; 9.6; 3.1)	1.48 ddt [0.04] (12.0 (2 ×); 11.5)

TABLE I
(*Continued*)

Proton	Compound *IV*			Compound *XXI*		
	$CDCl_3$	$CDCl_3 + C_6D_6$ (1:1)	+ TAI	$CDCl_3$	$CDCl_3 + C_6D_6$ (1:1)	+ TAI
H-8α	2.38 ddd (12.3; 4.1; 3.4)	2.06 dt (12.4; 4.0; 3.7)	2.16 dt [0.10] (12.1; 3.9; 3.6)	2.40 ddd (12.4; 4.2; 3.0)	2.08 ddd (12.3; 4.3; 3.1)	2.18 ddd [0.10] (12.0; 4.2; 3.1)
H-8β	1.34 dt (12.4; 12.2; 11.0)	1.00 dt (12.4; 12.2; 10.8)	1.24 q [0.24] (12.2; 12.1; 11.5)	1.34 dt (12.4; 12.4; 11.0)	1.02 dt (12.4; 12.3; 10.9)	1.25 q [0.23] (12.0 (2 ×); 11.5)
H-9	4.07 bdd (11.0; 3.8)	3.66 bdd (10.8; 4.1)	4.84 dd [1.18] (11.5; 3.9)	4.00 bdd (11.4; 4.2)	3.60 bdd (10.9; 4.3)	4.79 dd [1.19] (11.5; 4.2)
H-11	2.23 dq (12.0; 7.0 (3 ×))	1.84 dq (12.0; 7.0 (3 ×))	1.88 dq [0.04] (12.0; 7.0 (3 ×))	2.29 dq (12.0; 7.0 (3 ×))	1.91 dq (12.0; 7.0 (3 ×))	1.93 dq [0.02] (12.0; 7.0 (3 ×))
H-13	1.24 d (7.0)	1.06 d (7.0)	1.09 d [0.03] (7.0)	1.25 d (7.0)	1.08 d (7.0)	1.11 d [0.03] (7.0)
H-14	5.15 d (1.5)	4.97 d (1.6)	5.01 d [0.04] (1.6)	5.22 d (1.5)	5.04 d (1.5)	5.10 d [0.06] (1.3)
H-14′	5.49 b	5.31 b	5.35 b [0.04]	5.53 t (1.5; 1.1)	5.34 t (1.5; 1.0)	5.39 b [0.05]
H-15	1.20 d (6.2)	1.11 d (6.6)	1.15 d [0.04] (6.6)	0.97 d (7.2)	0.85 d (7.3)	0.95 d [0.10] (7.0)

TABLE II

^{1}H NMR parameters of guaianolide derivatives *X*, *XI*, and *XIX*. The values of coupling constants are given in parentheses; TAI-induced acylation shifts are given in square brackets

Proton	*X*	*XI*	*XIX*
H-1	3.00 q [0.02] (8.3 (3 ×))	2.99 q (8.7; 8.0 (2 ×))	2.88 m
H-2	1.75 – 1.90 m	1.75 – 1.90 m	2.43 dt (14.2; 8.4 (2 ×))
H-2′	1.75 – 1.90 m	1.75 – 1.90 m	1.82 ddd (14.2; 5.9; 4.6)
H-3	2.49 m [0.02]	2.46 m	4.60 ddt [1.10] (8.4; 6.0; 2.3; 2.0)
H-5	2.80 bdd [0.04] (10.4; 8.7)	2.82 bdd (10.3; 8.7)	2.80 m
H-6	3.97 dd [0.08] (10.4; 9.0)	4.02 dd (10.3; 8.9)	3.97 t [0.04] (9.8 (2 ×))
H-7	2.84 tt [0.39] (9.0 (2 ×); 3.5; 3.2)	3.14 tt (10.0; 8.9; 3.5; 3.0)	1.92 ddt (12.3; 11.9; 9.7; 3.0)
H-8	3.93 dt [1.16] (9.1; 5.3; 4.8)	4.98 dt (10.2; 5.4; 5.1)	2.44 ddd (12.4; 4.8; 3.1)
H-8′	–	–	1.33 dt (12.3 (2 ×); 10.6)
H-9	2.70 dd [0.12] (13.8; 5.3)	2.69 dd (14.2; 5.4)	4.14 bdd [1.03] (10.8; 4.8)
H-9′	2.28 dd [0.20] (13.8; 4.8)	2.31 ddd (14.2; 5.1; 0.7)	–
H-11	–	–	2.25 dq (11.9; 7.0 (3 ×))
H-13	6.27 dd [0.03] (3.5; 0.9)	6.25 d (3.5)	1.26 d [0.02] (7.0)
H-13′	6.16 dd [–0.41] (3.2; 0.9)	5.66 d (3.0)	–
H-14	5.05 b [0.06]	5.05 b	5.44 t [0.00] (1.3 (2 ×))
H-14′	4.96 b [0.03]	4.94 d (0.07)	5.21 b [0.01]
H-15	5.29 m [0.01] (2.5; 1.5 (2 ×); 1.0)	5.30 m (2.6 (2 ×); 2.0; 1.9)	5.35 t [0.17] (2.3; 2.0)
H-15′	5.09 m [0.02] (2.8; 2.0 (2 ×); 1.0)	5.10 m (2.1 (2 ×); 2.0; 0.9)	5.31 t [0.16] (2.2; 2.0)
OAc	–	2.15 s	–

absolute configuration of lactones *X* and *XI* was found to be 8(*S*), as derived from the sense of the difference in molecular rotations of the acetate *XI* and alcohol *X* on application of the Brewster rule[22].

In the species *V. menthaefolia* we found three lactones, *XIV* – *XVI*. The first, lactone *XIV*, melted at 188 – 192 °C, $[\alpha]_D$ 0°, composition $C_{24}H_{32}O_{11}$. Its IR spectrum showed the presence of a hydroxyl (3 595 and 3 460 cm^{-1}), an acetate (1 740 and 1 235 cm^{-1}), a γ-lactone (1 760 cm^{-1}), an unsaturated ester (1 710 and 1 637 cm^{-1}) and a methoxy group (2 820 cm^{-1}). The mass spectrum exhibited molecular peak M 496 and characteristic fragments 481 (M – CH_3), 464 (M – CH_3OH), 446 (464 – 18), 404 (464 – 60), 378 (464 – C_3H_5COOH), 350 (M – C_3H_5COOH – 60), 318 (378 – 60), 276 (318 – CH_2=CO), 258 (318 – 60) and 69 (C_3H_5CO). CD spectrum had a maximum at 205 nm (Δε –7.4) and 240 nm (Δε +9.2). The ^{1}H NMR spectrum (Table III) proved two methyl groups at tertiary carbon atoms of the type C(OR)CH_3 (δ 1.39 and 1.70 s), two acetate groups (δ 1.95 s and 2.16 s), a methoxy group (δ 3.36 s) and a methacrylate moiety (=CH_2: 5.98 p and 5.62 p, CH_3: 1.91 dd). Further we identified an isolated –CH_2O– group (δ 4.53 d and 4.26 d, J = 12.2 Hz), two protons of the type CH–OCOR of which one is isolated (δ 5.86 s) and the second is adjacent to a CH_2 group (δ 5.77 dd, J = 4.3 and 2.3 Hz) and, finally, an isolated –CH_2–CH_2– fragment. Comparison of the elemental formula and the proton NMR data indicated the presence of two OH groups of which only one was confirmed by in situ TAI-acylation (Table III). The ^{13}C NMR spectrum (Table IV) confirmed the above-mentioned structural fragments and, in addition, proved one tetrasubstituted double bond (δ 157.67 and 129.42). Detailed analysis of the NMR spectral data led us to suggestion of structure *XIV* whose acetate was later described and its structure solved[16]. The nonreactivity of the hydroxy group in position 1, which we observed in the TAI-acylation, was reported by the German authors[16] for classical acetylation and was explained by steric hindrance.

Another hitherto undescribed lactone of this kind, isolated in the present study, was lactone *XV*, m.p. 180 – 183 °C, $[\alpha]_D$ +30.3°, composition $C_{25}H_{34}O_{11}$. Its IR spectrum showed the presence of a hydroxyl (3 585 and 3 450 cm^{-1}), a γ-lactone (1 758 cm^{-1}), an acetate (1 738 cm^{-1} and 1 235 cm^{-1}) and a conjugated ester (1 710 sh and 1 634 cm^{-1}). In addition to the molecular peak (510), the mass spectrum exhibited characteristic fragments 481 (M – C_2H_5), 464 (M – C_2H_5OH), 450 (M – 60), 446 (464 – 18), 432 (450 – 18), 404 (464 – 60), 378 (464 – C_3H_5COOH), 318 (378 – 60), 276 (318 – CH_2=CO), 258 (318 – 60), 69 (C_3H_5CO), 43 (CH_3CO). CD spectrum contained maxima at 210 nm (Δε –4.5) and 240 nm (Δε +10.0). Comparison of the ^{1}H NMR spectra showed that the lactone *XV* differs from lactone *XIV* only in that it contains an ethoxy instead of a methoxy group in position 13 (CH_3: 1.17 t; CH_2O: 3.51 q, J = 7.0 Hz). As follows from comparison of NMR spectra, both the mentioned lactones *XIV* and *XV* were prepared last year synthetically from glaucolide A (*VII*) but no characteristic chiroptical data were reported for them[23].

The last of the three mentioned lactones isolated from *V. menthaefolia* species was noncrystalline lactone *XVI*, $[\alpha]_D$ +124.0°, composition $C_{20}H_{26}O_8$. According to IR spectrum, it contains a hydroxyl (3 550 cm^{-1}), a methoxyl (2 820 cm^{-1}), a γ-lactone (1 758 cm^{-1}), a conjugated ester (1 721 and 1 637 cm^{-1}) and a double bond (1 608 and

TABLE III

^{1}H NMR parameters of compounds *XIV – XVI* and *XX*. The coupling constants are given in parentheses; TAI-induced acylation shifts for 4-OTAC-derivatives *XIV* and *XV* and 4,10-diOTAC derivative *XVI* are given in square brackets

Proton	*XIV*	*XV*	*XVI*	*XX*
H-2	2.37 ddd [0.05]	2.38 ddd [0.05]	2.44 dt	2.42 dt
	(14.5; 4.5; 3.5)	(14.5; 4.5; 3.5)	(12.4; 12.4; 7.1)	(12.4; 12.4; 7.1)
H-2′	1.70 ddd [0.05]	1.68 ddd [0.05]	[a]	[a]
	(14.5; 12.5; 5.0)	(14.5; 12.5; 5.0)		
H-3	2.34 ddd [0.27]	2.32 ddd [0.27]	[a]	[a]
	(14.5; 12.5; 4.5)	(14.5; 12.5; 4.5)		
H-3′	1.86 ddd [0.75]	1.87 ddd [0.75]	[a]	[a]
	(14.5; 5.0; 3.5)	(14.5; 5.0; 3.5)		
H-5	5.86 s [0.31]	5.86 s [0.30]	5.85 bs [0.17]	5.87 bs
H-8	5.77 dd [–0.04]	5.83 dd [–0.05]	6.57 bd	6.42 bd
	(4.3; 2.3)	(4.3; 2.2)	(10.5; <2)	(10.5; <2)
H-9	3.46 dd [–0.01]	3.45 dd [–0.01]	2.59 dd	2.53 dd
	(16.0; 2.3)	(16.0; 2.2)	(16.2; 10.5)	(16.0; 9.6)
H-9′	2.10 dd [0.03]	2.09 dd [0.01]	2.11 bd	[a]
	(16.0; 4.3)	(16.0; 4.3)	(16.2; <2)	
H-13	4.53 d [0.02]	4.55 d [0.03]	4.58 bd [–0.31]	4.52 d
	(12.2)	(12.4)	(12.1)	(12.2)
H-13′	4.26 d [0.00]	4.31 d [–0.01]	4.28 d [-0.10]	4.31 d
	(12.2)	(12.4)	(12.1)	(12.2)
H-14	1.70 s [0.02]	1.71 s [0.01]	1.58 s [0.00]	1.58 s
H-15	1.39 s [0.46]	1.40 bs [0.45]	1.23 s [0.42]	1.22 s
OAc	1.95 s [–0.03]	1.95 s [–0.03]	–	2.11 s
	2.16 s [–0.03]	2.17 s [–0.00]		
OMe	3.36 s [0.08]	–	3.41 s [–0.06]	–
OEt	–	3.51 q [–0.01]	–	3.56 q
	–	1.17 t [0.00]	–	1.22 t
$OCOC(CH_3)=CH_2$	5.98 p [0.01]	5.98 p [0.01]	6.29 p [–0.04]	–
	5.62 p [–0.01]	5.61 p [0.00]	5.68 p [0.07]	–
	1.91 dd [0.02]	1.91 dd [0.02]	1.96 dd [0.01]	–

[a] Proton signal was not assigned.

1 655 cm^{-1}). Mass spectrum exhibited molecular peak M 394 and characteristic fragments 362 (M – CH_3OH), 344 (362 – 18), 320 (362 – CH_2=CO), 308 (M – C_3H_5COOH), 276 (308 – CH_3OH), 234 (276 – CH_2=CO), 216 (234 – 18) and 69 (C_3H_5CO). In the CD spectrum there were three maxima: at 210 nm ($\Delta\varepsilon$ –9.0), 255 nm ($\Delta\varepsilon$ +10.0) and at 318 nm ($\Delta\varepsilon$ –0.6). ^{1}H NMR spectrum (Table III) showed the presence of two tertiary methyl groups (singlets at δ 1.23 and 1.58), a methacrylate moiety (=CH_2: 6.29 p and 5.68 p; CH_3: 1.96 dd), a methoxy group (δ 3.41) and two hydroxy groups (broadened signals at δ 4.10 and 3.84, disappearing on addition of D_2O). In the lowfield region of the spectrum we further assigned signals to a CH–O proton in position 8 (broad doublet at δ 6.57 belonging to an isolated ABX system with CH_2 protons in position 9 at δ 2.59 dd and 2.11 bd), to an isolated olefinic proton in position 5 (δ 5.85 bs) and protons of isolated CH_2–O group in position 13 (δ 4.58 bd and 4.28 d, J = 12.1 Hz). Our proton NMR data and the structure *XVI* derived from them are in accord with the data described later[16].

In addition to compounds *I*, *V*, *XVII* and *XVIII*, obtained already earlier from other plant species, we isolated from species *V. moaensis* a hitherto undescribed noncrystalline lactone *XIX*, $[\alpha]_D$ +39.9°, composition $C_{15}H_{20}O_4$. Its IR spectrum indicated the presence of a hydroxyl (3 605 and 3 470 cm^{-1}), a γ-lactone (1 770 cm^{-1}) and a double bond (1 645 cm^{-1}). Mass spectrum contained molecular peak M 264 and characteristic

TABLE IV
Carbon-13 chemical shifts of compounds *XIV* and *XV*

Carbon	*XIV*	*XV*	Carbon	*XIV*	*XV*
1	89.20	88.84	14	19.17	19.66
2	29.35	30.78	15	22.89	23.44
3	35.29	35.89	OMe	58.04	–
4	72.71	73.20	OEt	–	66.36
5	75.73	76.43		–	15.11
6	~77.00[a]	~77.00[a]	OAc	171.59	171.67
7	157.67	157.14		171.05	171.26
8	65.95	66.17		22.77	23.37
9	33.87	34.20		19.90	20.39
10	84.18	84.25	OCOC(CH_3)=CH_2	167.47	167.64
11	129.42	130.53		135.89	136.33
12	160.07	169.12		125.39	125.81
13	62.66	61.44		17.68	18.20

[a] The signal is overlapped with a strong signal of $CDCl_3$.

fragments 246 (M – 18), 231 (M – 18 – CH_3), 218 (246 – CO) and 200 (218 – 18). CD maximum was located at 220 nm ($\Delta\varepsilon$ +0.7). ^{1}H NMR spectrum (Table II) proved the presence of a secondary methyl group (δ 1.26 d, J = 7.0 Hz), two exomethylene groups in position 14 and 15 (δ 5.44 t, 5.21 bs, 5.35 t and 5.31 t) and CH–O protons in positions 3, 6 and 9 (δ 4.60 ddt, 3.97 t and 4.14 bdd). The presence of hydroxyl groups in positions 3 and 9 was proved by in situ TAI-acylation (induced TAI-acylation shifts 1.10 and 1.03 ppm, respectively, see Table II). Detailed analysis of the NMR data (particularly coupling constants) led us to the structure *XIX*. Last year, lactone of the structure *XIX* was found in the aerial parts of species *Venidium fastuosum* STAP. (*Asteraceae*, *Arctotae*)[18] and its physical constants agree with our data.

So far undescribed was also the noncrystalline lactone *XX*, $[\alpha]_D$ +48.1°, composition $C_{19}H_{26}O_8$. According to IR spectrum, it contained a hydroxy group (3 535 cm^{-1}), an ethoxy group (2 860 cm^{-1}), a γ-lactone (1 755 cm^{-1}) and a double bond (1 653, 1 636 and 1 604 cm^{-1}). Mass spectrum exhibited molecular peak M 382 and characteristic fragments 336 (M – C_2H_5OH), 322 (M – 60), 294 (336 – CH_2=CO), 276 (336 – 60), 234 (276 – 42) and 216 (234 – 18). CD maxima were located at 250 nm ($\Delta\varepsilon$ +2.2) and 310 nm ($\Delta\varepsilon$ –0.7). Its ^{1}H NMR spectrum showed marked structural similarity with lactone *XVI*, except for substituents in positions 8 and 13 which in lactone *XX* are acetate (δ 2.11 s) and ethoxy group (δ 1.22 t and 3.56 q, J = 7.0 Hz), respectively, instead of methacrylate and methoxy groups in the lactone *XVI*.

In the present study we describe the isolation and structure determination of a series of sesquiterpenic lactones from four species of the *Vernonia* genus that are endemic in Cuba. The basic skeletons of these lactones (eudesmanolide, guaianolide (including the grandolide type), germacranolide (with hirsutinolide and glaucolide type specification) and cadinanolide (with vernojalcanolide type specification)) do not differ from those of sesquiterpenic lactones obtained from *Vernonia* species native in the New World (see e.g. refs[9,16]). From the viewpoint of chemosystematics of genus *Vernonia*, the Cuban endemites can be included among other species of the mentioned genus that are native particularly in Central and South America.

The sesquiterpenic lactones of the hirsutinolide and cadinanolide type, which had been described already by several authors as constituents of some species of the genus *Vernonia* and which were isolated also by us from several Cuban *Vernonia*, are regarded[23] as artifacts; this could have some implications in chemosystematics, biogenesis, etc. This opinion is based on the fact[23] that these hirsutinolides and cadinanolides were prepared from glaucolide A (*VII*) by treatment with methanol or ethanol in the presence of silica gel or alumina. However, we think that the cited results cannot serve as unequivocal arguments for regarding hirsutinolides and cadinanolides as artifacts. In a living plant organism, native compounds of the glaucolide A type (*VII*) may undergo many enzymatic reactions leading to products identical with those obtained by other mechanisms, e.g. by a chemical way. Moreover, in the processing of the aerial part of

species *V. menthaefolia* we did not use ethanol at all and methanol was employed only in the last stage of the chromatography when both hirsutinolide *XVI* and cadinanolides *XIV* and *XV* had been already eluted with less polar solvents. Similarly, although the mentioned solvents were not used at all in the work-up of the aerial parts of *V. moaensis*, we isolated hirsutinolide *XX*. We therefore assume that there is no proof of the artifact character of hirsutinolides and cadinanolides found in species of *Vernonia* genus.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer PE 580 spectrometer in chloroform solution unless stated otherwise; wavenumbers are given in cm^{-1}. Optical rotations were measured in methanol, unless stated otherwise, on objective polarimeter Perkin–Elmer 141. Mass spectra were measured on a ZAB-EQ V.G. Analytical spectrometer (Manchester, U.K.); electron impact, 70 eV. CD spectra (nm, $\Delta\varepsilon$, methanol) were measured on an autodichrograph Jobin–Yvonne Mark V. ^{1}H NMR spectra were taken in deuteriochloroform (with tetramethylsilane as internal reference) on a Varian XL-200 (at 200 MHz) and/or Varian UNITY-500 (at 500 MHz) FT NMR spectrometer. 2D-COSY spectra were used for the assignment of coupled protons. Interproton NOE's were determined from difference 1D-NOE spectra: first spectrum was run with selective irradiation of given proton during time-period (6 s) before the acqusition time and then reference spectrum (taken under the same conditions but with off-resonance decoupling frequency setting) was acquired and substracted from the first one to give difference NOE spectrum. In situ TAI-acylation[24] was done by the addition of small excess of TAI to the $CDCl_3$ solution of compound in NMR sample tube and product was characterized by ^{1}H NMR spectrum. ^{13}C NMR spectra were run in the same solvent on the Varian UNITY-500 (at 125.7 MHz) spectrometer, using "attached proton test" pulse sequence for the classification of carbons by the number of directly bound protons.

Isolation of Constituents

The known compounds – lupeol acetate (*I*), scopoletin (*II*), lupeol palmitate (*VI*), velutin (*XII*) and genkwanin (*XIII*) – were identified on the basis of their spectral properties (IR, MS, UV, ^{1}H NMR), melting points and specific rotation.

a) From *Vernonia angusticeps*. Aerial parts of species *Vernonia angusticeps* EKM., collected in April 1980 at San Piedra, Santiago de Cuba (voucher HAJB 41577 is deposited in Herbarium of the National Botanical Garden in Habana), were dried and finely ground (900 g). As shown by IR spectrum, light petroleum extract of this material contained no compounds with a γ-lactone ring. Further extraction of the mentioned material with chloroform and evaporation of the solvent afforded a residue (15 g) which was chromatographed on a column of silica gel (400 g). Elution with toluene–diethyl ether (9 : 1) gave lupeol acetate (*I*), m.p. 208 – 210 °C (acetone) and $[\alpha]_D$ +45.7° (*c* 0.35, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$): 0.78, 0.84, 0.85, 0.93, 1.03 s (5 × CH_3); 1.6 dd (CH_3–C=, J = 1.3 and 0.7 Hz); 2.04 s (OAc); 2.38 dt (H-19, J = 11, 11.5 and 5.0 Hz); 4.47 dd (H-3, J = 11.0 and 5.0 Hz); 4.57 m (H-29); 4.69 m (H-29′). Mass spectrum (*m*/*z*): 468 (M), 408 (M – 60). IR spectrum (cm^{-1}): 1 722, 1 254 (acetate); 1 639 (double bond). Elution with toluene–diethyl ether (4 : 1), followed by preparative thin-layer chromatography, afforded scopoletin (*II*), m.p. 195 – 197 °C (methanol). UV spectrum (nm): 227, 296, 394. Mass spectrum (*m*/*z*): 192 (M). Elution with toluene–diethyl ether (6 : 4) gave 3-oxograndolide (*III*), m.p. 139 – 143 °C (ethyl acetate) and $[\alpha]_D$ +93°

(*c* 0.35). Its IR, ^{1}H NMR, mass and CD spectra were identical with those of the described compound[3 – 5] and no mixture melting point depression was observed. Further elution with ethyl acetate–diethyl ether (1 : 1) furnished 3β-hydroxygrandolide (*IV*), m.p. 162 – 165 °C, $[\alpha]_D$ +6.5° (*c* 0.42), composition $C_{15}H_{22}O_4$. IR spectrum (cm^{-1}): 1 768 (γ-lactone); 3 610 and 3 490 (hydroxyl); 1 643 (double bond). Mass spectrum (*m/z*): 266 (M), 248 (M – 18), 230 (M – 18 – 18). CD spectrum: 216, +1.1. For ^{1}H NMR spectrum see Table I. For $C_{15}H_{22}O_4$ (266.3) calculated: 67.64% C, 8.33% H; found: 67.58% C, 8.47% H. Ethyl acetate eluted reynosin (*V*), m.p. 140 – 143 °C, $[\alpha]_D$ +135.5° (*c* 0.28). Its identity with a standard[6 – 8] was proved by comparison of ^{1}H NMR, IR, CD, and mass spectra and mixture melting point.

b) From *Vernonia acunnae*. Aerial parts of species *V. acunnae* ALAIN., collected in April 1980 in the region of river Yagrumaje, Moa, Oriente (voucher HAC 29170 is deposited in Herbarium of the Cuban Academy of Sciences, Habana), were dried and finely ground (580 g). On extraction with light petroleum and the usual work-up, this material afforded a sirupy residue (21 g) which was chromatographed on a column of silica gel (500 g). Elution with light petroleum gave lupeol palmitate (*VI*), m.p. 74 – 77 °C, $[\alpha]_D$ +28.9° (*c* 0.35, $CHCl_3$). ^{1}H NMR spectrum ($CDCl_3$): signals of the lupeol part identical with lupeol acetate (vide supra); 0.88 t (CH_3, *J* = 6.5 Hz); 1.25 bs ($(CH_2)_n$), 2.29 t ($COCH_2$). Mass spectrum (*m/z*): 664 (M), 409 (M – $C_{15}H_{31}COO$). Elution with light petroleum–toluene (1 : 1) afforded lupeol acetate (*I*), identical in all respects with compound obtained from *V. angusticeps*. Toluene–ethyl acetate (4 : 1) eluted glaucolide A (*VII*), m.p. 152 – 155 °C, $[\alpha]_D$ –13.0° (*c* 0.36). Its ^{1}H NMR, IR and mass spectra corresponded to the literature data[10]. Further elution with toluene–ethyl acetate (4 : 1), followed by preparative thin-layer chromatography, afforded noncrystalline piptocarphin A (*VIII*), $[\alpha]_D$ +71.0° (*c* 0.6, $CHCl_3$). ^{1}H NMR, IR and mass spectrum were identical with the published data[11]. After extraction with light petroleum, the plant material was extracted with chloroform. The sirupy residue (10 g), obtained on evaporation of the solvent, was column chromatographed on silica gel (250 g). Elution with toluene–chloroform (1 : 1) afforded 1α*H*,5α*H*,6β*H*,7α*H*,8α-acetoxyguai-4(15),10(14),11(13)-trien-6,12-olide (*XI*), m.p. 112 – 114 °C (ethyl acetate–light petroleum), $[\alpha]_D$ +78.8° (*c* 0.34). IR spectrum: 1 763 (γ-lactone); 1 739, 1 245 (acetate); 1 643, 1 657 (double bond). Mass spectrum (*m/z*): 288 (M), 246 (M – CH_2=CO), 228 (M – 60), 148, 91, 43. CD spectrum: 220, –3.8. For $C_{17}H_{20}O_4$ (288.3) calculated: 70.83% C, 6.94% H; found: 70.69% C, 6.85% H. For ^{1}H NMR spectrum see Table II. Elution with chloroform gave tulipinolide (*IX*), m.p. 178 – 182 °C, $[\alpha]_D$ +231.0° (*c* 0.51, $CHCl_3$). Its ^{1}H NMR, IR and mass spectra were in accord with the published ones[12,13]. Chloroform–methanol (99 : 1) washed out 1α*H*,5α*H*,6β*H*,7α*H*,8α-hydroxyguai-4(15),10(14),11(13)-trien-6,12-olide, (*X*), m.p. 92 – 95 °C, $[\alpha]_D$ +57.2 (*c* 0.45, acetone). Its ^{1}H NMR (Table II), IR and mass spectrum corresponded to the literature values[15].

c) From *Vernonia menthaefolia*. The aerial parts of species *V. menthaefolia* (PAPP. ex SPRENG.) LESS., collected in April 1980 at Gran Piedra, Santiago de Cuba (voucher HAJB 41576 is deposited at Herbarium of the National Botanical Garden, Habana), were dried and finely ground (800 g). Extraction with light petroleum and the usual processing afforded a sirupy residue (18 g) which was chromatographed on a column of silica gel (500 g). Elution with toluene–ethyl acetate (4 : 1) gave crystalline velutin (*XII*), m.p. 228 – 233 °C (ethyl acetate–toluene). UV spectrum (nm): 235, 255, 340. Mass spectrum (*m/z*): 314 (M), 167 ($C_8H_7O_4$), 148 ($C_9H_8O_2$). ^{1}H NMR spectrum ($CDCl_3$): 3.89 s and 4.01 s (2 × OCH_3); 6.38 d (H-6, *J*(6,8) = 2.3 Hz); 6.49 d (H-8, *J*(8,6) = 2.3 Hz); 6.57 s (H-3); 7.04 d (H-5′, *J*(5′,6′) = 8.4 Hz); 7.34 d (H-2′, *J*(2′,6′) = 2.1 Hz); 7.49 dd (H-6′, *J*(6′,5′) = 8.4 and *J*(6′,2′) = 2.1 Hz). Further elution with the same solvent mixture gave genkwanin (*XIII*), m.p. 286 – 289 °C. ^{1}H NMR spectrum (CD_3SOCD_3): 3.87 s (OCH_3); 6.39 d (H-6, *J*(6,8) = 2.3 Hz); 6.77 d (H-8, *J*(8,6) = 2.3 Hz); 6.85 s (H-3); 6.94 m (H-3′ and H-5′); 7.96 m (H-2′ and H-6′). Elution with toluene–ethyl acetate (3 : 2) followed by preparative thin-layer chromatography, furnished noncrystalline 1α,10α-

dihydroxy-8α-(2′-methyl)acryloyloxy-13-methoxyhirsutinolide (*XVI*), $[\alpha]_D$ +124.0° (*c* 0.38), composition $C_{20}H_{26}O_8$. IR spectrum: 3 550 (hydroxyl); 2 820 (methoxyl); 1 758 (γ-lactone); 1 721 (α,β-unsaturated ester); 1 655, 1 637, 1 608 (double bond). Mass spectrum (*m*/*z*): 394 (M), 362 (M – CH_3OH), 344 (362 – 18), 320 (362 – CH_2=CO), 308 (M – C_3H_5COOH), 276 (308 – CH_3OH), 234 (276 – CH_2=CO), 216 (234 – 18), 148, 99, 69 (C_3H_5CO). CD spectrum: 210, –9.0; 255, +10.0; 318, –0.6. For ^{1}H NMR spectrum see Table III. For $C_{20}H_{26}O_8$ (394.4) calculated: 60.90% C, 6.65% H; found: 61.25% C, 6.84% H. Toluene–ethyl acetate mixture (2 : 3) washed out material (3.1 g) which was rechromatographed on silica gel (300 g). The first ethyl acetate fractions gave 8α-(2′-methyl)acryloyloxy-13-ethoxyvernojalcanolide (*XV*), m.p. 180 – 183 °C (acetone–ethyl acetate), $[\alpha]_D$ +30.3° (*c* 0.6, acetone), composition $C_{25}H_{34}O_{11}$. IR spectrum: 3 585, 3 450 (hydroxyl); 1 758 (γ-lactone); 1 738, 1 235 (acetate); 1 710 sh (α,β-unsaturated ester); 1 634 (double bond). Mass spectrum (*m*/*z*): 510 (M), 481 (M – C_2H_5), 464 (M – C_2H_5OH), 450 (M – 60), 446 (464 – 18), 432 (450 – 18), 404 (464 – 60), 378 (464 – C_3H_5COOH), 318 (378 – 60), 276 (318 – CH_2CO), 258 (318 – 60), 99, 69 (C_3H_5CO), 43 (CH_3CO). CD spectrum: 210, –4.5; 240, +10.0. For ^{1}H and ^{13}C NMR spectral data see Tables III and IV. For $C_{25}H_{34}O_{11}$ (510.5) calculated: 58.81% C, 6.71% H; found: 58.69% C, 6.92% H. The last ethyl acetate fractions afforded 8α-(2′-methyl)acryloyloxy-13-methoxyvernojalcanolide (*XIV*), m.p. 188 – 192 °C (ethyl acetate), $[\alpha]_D$ 0° (*c* 0.29), composition $C_{24}H_{32}O_{11}$. IR spectrum: 3 595, 3 460 (hydroxyl); 2 820 (methoxyl); 1 760 (γ-lactone); 1 740, 1 235 (acetate); 1 710 (α,β-unsaturated ester); 1 637 (double bond). Mass spectrum (*m*/*z*): 496 (M), 481 (M – CH_3), 464 (M – CH_3OH), 446 (464 – 18), 418 (M – 18 – 60), 404 (464 – 60), 378 (464 – C_3H_5COOH), 350 (M – C_3H_5COOH – 60), 318 (378 – 60), 276 (318 – 42), 258 (318 – 60), 99, 69 (C_3H_5CO), 43 (CH_3CO). CD spectrum: 205, –7.4; 240, +9.2. For ^{1}H and ^{13}C NMR spectra see Tables III and IV. For $C_{24}H_{32}O_{11}$ (496.5) calculated: 58.05% C, 6.50% H; found: 57.87% C, 6.64% H.

d) From *Vernonia moaensis*. Aerial parts of species *V. moaensis* ALAIN., collected in April 1980 in the region of river Yagrumaje, Moa, Oriente (voucher HAC 29169 is deposited at Herbarium of the Cuban Academy of Sciences, Habana), were dried and finely ground (750 g). The usual processing of the light petroleum extract afforded a sirupy residue (16 g) which was column chromatographed on silica gel (400 g). Light petroleum–benzene (1 : 1) eluted lupeol palmitate (*VI*) and lupeol acetate (*I*). Both compounds *I* and *VI* were identical with the analogous compounds obtained from *V. anguaticeps* and *V. acunnae*. Elution with benzene–chloroform (1 : 1) afforded costunolide (*XVII*), m.p. 102 – 106 °C, $[\alpha]_D$ +115.2° (*c* 0.5, chloroform). Its IR, ^{1}H NMR, CD and mass spectra corresponded to the published data[25]. Subsequent extraction of the plant material with chloroform, followed by usual work-up, gave a sirupy residue (12 g) which was chromatographed on a column of silica gel (300 g). Elution with benzene–chloroform (4 : 1) and repeated preparative thin-layer chromatography furnished noncrystalline dihydrodehydrocostuslactone (*XVIII*), $[\alpha]_D$ +7.3° (*c* 0.4, chloroform). Its ^{1}H NMR, IR and mass spectra were in accord with the published data[17]. Elution with benzene–chloroform (1 : 1) and repeated column and preparative thin-layer chromatography afforded noncrystalline 1α,10α-dihydroxy-8α-acetoxy-13-ethoxyhirsutinolide (*XX*) (75 mg), $[\alpha]_D$ +48.1° (*c* 0.5). IR spectrum: 3 535 (hydroxyl); 2 860 (ethoxyl); 1 755 (γ-lactone); 1 653, 1 636, 1 604 (double bond). Mass spectrum (*m*/*z*): 382 (M), 336 (M – C_2H_5OH), 322 (M – 60), 294 (336 – 42), 276 (336 – 60), 234 (276 – 42), 216 (234 – 18), 188, 148, 99. CD spectrum: 250, +2.2; 310, –0.7. For ^{1}H NMR spectrum see Table III. For $C_{19}H_{26}O_8$ (382.4) calculated: 59.67% C, 6.85% H; found: 59.79% C, 6.98% H. Elution with chloroform with 1% of methanol gave noncrystalline 3β-hydroxy-4,15-dehydrograndolide (*XIX*) (55 mg), $[\alpha]_D$ +39.9° (*c* 0.34). IR spectrum: 3 605, 3 470 (hydroxyl); 1 770 (γ-lactone); 1 645 (double bond). Mass spectrum (*m*/*z*): 264 (M), 246 (M – 18), 231, 218, 200, 173, 77. CD spectrum: 220, +0.7. For ^{1}H NMR spectrum see Table II. For $C_{15}H_{20}O_4$ (264.3) calculated: 68.16% C, 7.87% H; found: 68.07% C, 7.87% H.

Preparation of 3β-Hydroxygrandolide (*IV*) from 3-Oxograndolide (*III*)

A suspension of $NaBH_4$ (50 mg) in methanol (4 ml) was added to a solution of 3-oxograndolide (50 mg) in methanol (4 ml). After stirring at room temperature for 1 h, water (6 ml) was added and the mixture was acidified with 5% sulfuric acid. The product was taken up in chloroform, the extract was worked up as usual and the residue was purified by chromatography to afford 3β-hydroxygrandolide (*IV*, 20 mg), identical in all respects (including the mixture melting point) with a standard.

Preparation of Compound *X* from Lactone *XI*

A solution of Na_2CO_3 (200 mg) in water (10 ml) was added to a solution of lactone *XI* (60 mg) in methanol (10 ml). After standing at room temperature for 1 h, the mixture was acidified with 5% sulfuric acid and the product was extracted with chloroform. The chloroform extract was worked up in the usual manner and the residue (37 mg) was chromatographed on a thin layer of silica gel in chloroform–methanol (9 : 1) to give lactone *X*, identical in all respects with a standard. No mixed melting point depression was observed.

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