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CORYMBOTINS A–I: HIGHLY OXIDIZED KOLOVANE DERIVATIVES FROM *CASEARIA CORYMBOSA*

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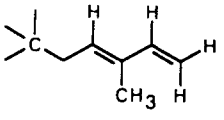
ABSTRACT.—A family of kolovane and norkolovane diterpenoids **6–14** has been isolated from leaves of the tropical tree *Casearia corymbosa*. These structures have been assigned based on data provided by various spectroscopic techniques, including a variety of 2D nmr experiments. Each of these nine compounds contains an unusual, highly oxidized C ring that may be viewed as a protected dialdehyde; the norkolovanes contain an additional aldehyde group as well.

Leafcutter ants (Hymenoptera, Formicidae, Attini) are considered by many to be the most serious agricultural pests in the tropical and subtropical Americas (1). Despite their voracious attack on a broad range of cultivated species, many plants native to their range escape any serious defoliation. During the course of our studies on natural plant defenses against leafcutter ants, we have examined a variety of plant species in diverse plant families (2–4). When field bioassays in Panama indicated that the plant *Casearia corymbosa* H.B.K. (Flacourtiaceae) is seldom attacked by *Acromyrmex octospinosus* (Reich) (Formicidae: Attini), a common Panamanian leafcutter, we began an investigation of the chemistry of this species. In this paper, we report the isolation and characterization of several terpenoids obtained from this plant, a family of new compounds **6–14** we have named the corymbotins.

RESULTS AND DISCUSSION

Air-dried leaves of *Casearia corymbosa* were extracted with $CHCl_3$, and the crude $CHCl_3$ extract was subjected to dry cc over Si gel. Two fractions of intermediate polarity, eluting with 25 and 45% EtOAc in hexane, respectively, contained an interesting array of downfield signals in their ^{1}H-nmr spectra and became the focus of our attention. These two fractions were further purified first by flash cc over Si gel and sub-



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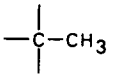

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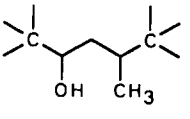

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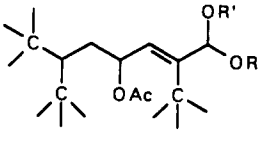

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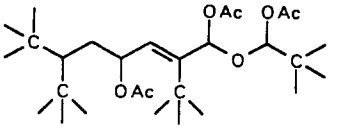

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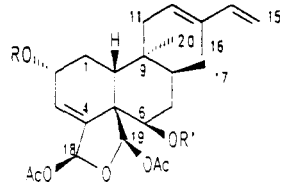
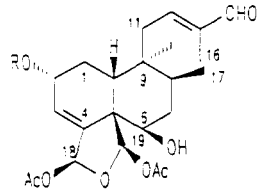


6 R=Ac, R′=Me
7 R=$(Me)_2$CHCO, R′=Me
8 R=H_2C=C(Me)CO, R′=Me
9 R=Ac, R′=H
10 R=$(Me)_2$CHCO, R′=H
11 R=H_2C=C(Me)CO, R′=H



12 R=Ac
13 R=$(Me)_2$CHCO
14 R=H_2C=C(Me)CO

sequently by reversed-phase hplc, affording a series of uv-active compounds named corymbotins A–I. Three compounds, corymobtins A [**6**], D [**9**], and G [**12**], were obtained in pure form through this sequence. The remaining compounds were obtained as three pairs (B/C [**7**,**8**], E/F [**10**,**11**], and H/I [**13**,**14**]) of isobutyrate/methacrylate mixtures. Because corymbotin D [**9**] was the most abundant pure compound, it was studied first.

Although the highest ion in the hrms of corymbotin D [**9**] was observed at *m/z* 416.2197, corresponding to a composition of $C_{24}H_{32}O_6$, the ^{13}C-nmr and DEPT spectra showed 26 carbons with multiplicities requiring a total of 35 -CH_n protons. The presence of three acetate moieties was apparent from the ^{13}C resonances, suggesting that the 416 ion corresponds to loss of one molecule of HOAc from the molecular ion. Together, these data indicate a molecular composition of $C_{26}H_{36}O_8$, including one -OH group and three acetate groups.

Both the ^{1}H- and ^{13}C-nmr spectra contained numerous well-separated and easily assigned resonances, but the carbon spectrum was particularly helpful since it contained 26 resonances spread evenly over a 160 ppm range. Based on this nmr data (Table 1), the nine degrees of unsaturation could be attributed to three carbon-carbon double bonds, three acetate carbonyl groups, and three ring systems. In addition to the three acetates, the DEPT experiment and the ^{1}H-nmr data revealed the presence of three methyl groups, four methylene units, and nine methine carbons. The remaining four carbons must be quaternary; shift data suggested that two were aliphatic and that two were olefinic.

With information obtained from a ^{1}H-^{1}H COSY experiment, these carbons could be assembled into partial structures **1**–**4**. Observation of couplings between one acetal proton and both a vinylic proton (δ 5.92) and a proton geminal to the acetate group reinforce the proposal of structure **4**. To be consistent with the molecular formula, both acetals must share one oxygen, allowing expansion of structure **4** to partial structure **5**.

A series of selective INEPT experiments (5) was employed to join these partial structures through the bridging quaternary carbons. In the key experiments (Table 1), the gross structure of the A ring was established by observation of a quaternary carbon

(53.34 ppm) upon irradiation of both one vinylic proton (δ 5.92) and the bridgehead methine (δ 2.38). The C ring was confirmed by observation of each acetal carbon upon irradiation of the opposite acetal proton, as well as by correlations between these protons and two A-ring carbons. The B ring was partially established by one set of experiments linking the bridgehead methine, a methyl singlet (δ 0.86), the methyl doublet (δ 0.93), and one vinylic proton (δ 5.37) to a single quaternary carbon (38.27 ppm). Experiments demonstrating the correlation of the proton geminal to the -OH group with both the quaternary sp^3 and sp^2 carbons of the A ring and one acetal carbon defined the remainder of this ring. These conclusions led to assigment of a kolovane skeleton to corymbotin D, as shown in structure **9**.

The relative stereochemistry of the eight chiral centers in corymbotin D was assigned on the basis of coupling constants and a NOESY experiment. The large coupling constants between H-10 and H-1 (14.1 Hz) and between H-2 and H-1 (9.5 Hz) indicated that both H-10 and H-2 were axial. Furthermore, diaxial coupling between H-6 and H-7 (12.1 Hz) required that the hydroxyl group must be equatorial. Homonuclear decoupling of the methyl doublet (C-17) indicated H-8 also had a large coupling con-

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Corymbotin D [**9**].

Position	^{13}C	^{1}H	Selective INEPT correlations[a]
1	26.01 (t)	1.71 (m) ax 2.18 (m) eq	
2	70.70 (d)	5.61 (m)	3, 4, 2-Ac
3	123.81 (d)	5.92 (br s)	1, 5, 18
4	144.32 (s)		
5	53.34 (s)		
6	73.85 (d)	3.99 (dd; 3.9, 12.1)	4, 5, 19
7	37.51 (t)	1.69 (m) ax 1.79 (m) eq	
8	36.67 (d)	1.83 (m)	
9	38.27 (s)		
10	41.28 (d)	2.38 (dd; 2.7, 14.1)	2, 5, 6, 7, 19
11	29.90 (t)	1.66 (dd; 1.5, 17.5) 2.20 (dd; 7.6, 17.5)	
12	128.63 (d)	5.37 (dd; 1.5, 7.6)	9, 14, 16
13	135.70 (s)		
14	141.01 (d)	6.30 (dd; 10.7, 17.3)	12, 13, 16
15	110.98 (t)	4.95 (d; 10.7) 5.10 (d; 17.3)	
16	11.87 (q)	1.66 (s)	
17	15.50 (q)	0.93 (d; 6.7)	7, 8, 9
18	95.10 (d)	6.70 (dd; 1.5, 1.5)	3, 18-Ac, 19
19	96.58 (d)	6.47 (s)	4, 18, 19-Ac
20	24.92 (q)	0.86 (s)	8, 9, 10, 11
Acetates			
2	170.68 (s)		
18	169.93 (s)		
19	169.30 (s)		
	21.10 (q)	2.08 (s)	
	21.10 (q)	2.08 (s)	
	21.54 (q)	1.94 (s)	

[a]Carbon resonances observed upon irradiation of the indicated ^{1}H resonance. The results from separate experiments optimized for different couplings are combined in this table.

stant (10.1 Hz) that required an equatorial methyl group. The NOESY experiment clearly showed nOe correlations among H-19, H_{ax}-7, and H-11, and between H-10 and H-12 (6). These through-space interactions are best accommodated by a cis A/B ring junction, an α H-19, and an equatorial methyl group at C-9 (Figure 1). The H-18 acetal proton was assigned an α configuration based on observation of an nOe between H-18 and H-19 as well as the allylic and homoallylic couplings between H-3 and H-18, H-2 and H-18 (7). Finally, an *E* configuration of the Δ^{12} olefin was supported by both the upfield shift for C-16 (11.87 ppm) and the nOe observed between H-12 and H-14. Together, these observations led to assignment of the relative stereochemistry shown in structure **9** to corymbotin D.

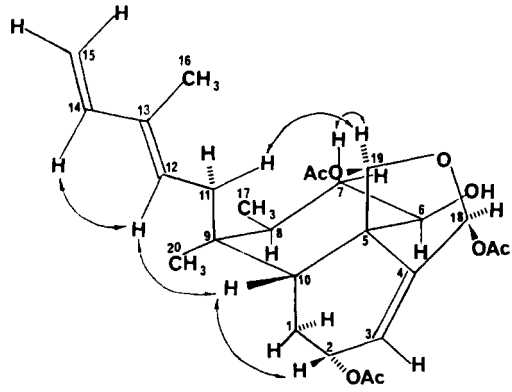


FIGURE 1. Selected NOESY correlations for corymbotin D [**9**].

The hrms of corymbotin A [**6**] revealed fragment ions at 458.2281 $[M - MeOH]^+$ and 430.2368 $[M - HOAc]^+$, corresponding to a molecular formula of $C_{27}H_{38}O_8$. Both the ^1H- and ^{13}C-nmr spectra for compound A (Table 2) were similar to those of corymbotin D, with an additional methoxy resonance presumably in place of the 6-OH group. The upfield shift for C-7 relative to compound **9** (31.51 vs. 37.51 ppm) supports a 6-methoxy connection, as does information from a COSY experiment. In particular, because H_{ax}-7 was shifted slightly upfield in corymbotin A relative to D, it was not obscured by overlap with the resonance of the vinylic methyl group. Strong coupling between H_{ax}-7 and the three protons assigned as H_{eq}-7, H_{ax}-6, and H_{ax}-8 was apparent in the ^1H-nmr spectrum of corymbotin A, allowing assignment of the relative stereochemistry at C-6 and C-8. The remaining stereogenic centers were assigned relative stereochemistry analogous to compound **9** through analysis of comparable coupling constants and nOe effects (Table 2). In addition, a correlation in the NOESY spectrum between the methoxy protons and H-18 further supports the α configuration assigned to H-18. These observations lead to assignment of structure **6** to corymbotin A.

Of the remaining corymbotins, only corymbotin G [**12**] was isolated in pure form. Corymbotin G was attributed a molecular composition of $C_{25}H_{34}O_9$ based on a fragment ion observed at *m/z* 418.1981 $[M - HOAc]^+$ in the hrms. Both ^1H- and ^{13}C-nmr spectra revealed the presence of an aldehyde carbonyl group and the absence of a terminal olefin in comparison with the nmr spectra of compound **9**. This suggested that corymbotin G had an aldehyde group in the side chain, presumably derived from degradation of the terminal olefin. The relative deshielding of C-12 (150.59 ppm) and H-12 (δ 6.47) clearly indicated an α,β-unsaturated aldehyde. Thus compound G was assigned a norkolovane skeleton as shown in structure **12**. The significant upfield shift of C-16 (9.53 ppm) indicated an *E* configuration of the side chain double bond. Relative

TABLE 2. ^{1}H- and ^{13}C-nmr Data For Corymbotin A [**6**] and G [**12**].

Position	Compound			
	6		**12**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	26.34 (t)	1.71 (m) ax 2.19 (m) eq	26.14 (t)	1.75 (m) ax 2.22 (m) eq
2	70.82 (d)	5.60 (m)	70.50 (d)	5.62 (m)
3	123.33 (d)	5.86 (br s)	124.13 (d)	5.94 (br s)
4	145.32 (s)		144.02 (s)	
5	52.77 (s)		53.36 (s)	
6	82.76 (d)	3.50 (dd; 3.9, 12.2)	73.75 (d)	4.03 (dd; 3.3, 12.2)
7	31.51 (t)	1.45 (m) ax 1.92 (m) eq	37.60 (t)	1.61 (m) ax 1.71 (m) eq
8	36.17 (d)	1.77 (m)	36.62 (d)	1.93 (m)
9	38.39 (s)		38.60 (s)	
10	41.41 (d)	2.39 (dd; 2.8, 14.0)	41.73 (d)	2.40 (dd; 2.1, 13.3)
11	29.90 (t)	1.68 (m) 2.21 (m)	31.30 (t)	1.87 (m) 2.42 (m)
12	128.95 (d)	5.37 (m)	150.59 (d)	6.47 (m)
13	135.52 (s)		141.22 (s)	
14	141.11 (d)	6.29 (dd; 10.7, 17.3)	194.10 (d)	9.42 (s)
15	110.91 (t)	4.93 (d; 10.7) 5.08 (d; 17.3)		
16	11.87 (q)	1.65 (s)	9.53 (q)	1.70 (s)
17	15.71 (q)	0.94 (d; 6.8)	15.57 (q)	0.97 (d; 6.8)
18	95.65 (d)	6.62 (dd; 1.6, 1.6)	95.13 (d)	6.71 (dd; 1.5, 1.5)
19	97.09 (d)	6.43 (s)	96.82 (d)	6.47 (s)
20	25.09 (q)	0.83 (s)	24.98 (q)	0.91 (s)
Acetates				
	170.69 (s)		170.70 (s)	
	170.16 (s)		169.91 (s)	
	169.41 (s)		169.02 (s)	
	21.20 (q)	2.09 (s)	21.17 (s)	2.09 (s)
	21.28 (q)	2.09 (s)	21.17 (s)	2.09 (s)
	21.77 (q)	1.93 (s)	21.70 (s)	1.93 (s)
OMe				
	57.46 (q)	3.34 (s)		

stereochemistry was determined by analysis of coupling constants and by a NOESY experiment, as described for **9**.

In addition to the three main compounds described above, a total of six related diterpenoids was isolated. These later compounds were separated into three pairs, but could not be readily isolated in pure form. Based on nmr analyses of the pairs, it is clear that the pairs corymbotins B/C, E/F, and H/I correspond to the C-2 isobutyrate and C-2 methacrylate esters analogous to compounds **6**, **9**, and **12**, respectively. In each case, the relative stereochemistry could be established by comparing nmr data with that of the coresponding pure compound.

All of these natural products contain a highly oxidized C ring that may be viewed as a protected dialdehyde, an array of functionality that has been found recently in a few related diterpenoids (6,8). Several similar compounds from *Zuelania guidonia* were described after these structures had been assigned (9,10), and related compounds from *C. corymbosa* stem bark were reported more recently (11). The corymbotins are also re-

miniscent of such biologically interesting compounds as polygodial and warburganal (12). Different kolovanes have a range of significant biological activities (13–17), including many that are potent insect antifeedants (18). The less abundant C-19 corymbotins may prove to be the more interesting members of this family. With the presence of the additional carbonyl group, these compounds represent doubly protected *tri*aldehydes, an oxidation state rarely observed in natural products. For these reasons, all of the corymbotins, but especially the C_{19} group, might be expected to demonstrate significant biological activity. Bioassays designed to measure their activity as leafcutter ant repellents (19), or as toxins either to the leafcutters or to the leafcutters' symbiotic fungus (20), are anticipated and will be reported in due course.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The nmr spectra (^1H and ^{13}C) were recorded on Bruker WM-360 or MSL-300 spectrometers as $CDCl_3$ solutions with an internal TMS standard. Carbon multiplicities were determined by DEPT experiments. All ^{13}C assignments are based on multiplicities, selective INEPT correlations, and/or comparisons within the compound series. Low resolution eims and high resolution eims were obtained on VG instrument TRIO-1 and ZAB-HF mass spectrometers, operating at 70 eV.

PLANT COLLECTION.—*C. corymbosa* leaves were collected on the Rodman Naval Ammunition Supply Depot (ca. 10 km west of Panama City, Panama), air-dried at ambient temperature, and stored in plastic bags until extracted. Voucher specimens (J.J. Howard #151) have been deposited at the National Museum, Panama City, Panama, and the Missouri Botanical Gardens, St. Louis, Missouri.

ISOLATION.—*C. corymbosa* leaves (120 g) were ground in a Waring blender, and then extracted with $CHCl_3$ using a Soxhlet extractor. After concentration of the $CHCl_3$ extract in vacuo, approximately 16 g of a residue remained. A portion of this residue (ca. 9 g) was separated into large fractions by dry cc on Si gel, eluting with hexane containing increasing amounts of EtOAc.

The fraction eluting with 25% EtOAc was subjected to flash cc over Si gel with an EtOAc/hexane gradient and finally purified by reversed-phase hplc (1 × 25 cm, C-18, 8 μm, uv monitor at 254 nm) eluting with 80% MeOH/H_2O. Six major components were obtained from this fraction, of which the first (corymbotin A, 30 mg) and fourth (corymbotin D, 60 mg) were pure. Corymbotins B and C (combined 30 mg) and E and F (125 mg total) were obtained as mixtures, each of which was very difficult to separate further.

The dry column fraction eluting with 45% EtOAc also was subjected to further purification first by flash cc and then by reversed-phase hplc. Corymbotin G (4.6 mg) was isolated in pure form while H and I were isolated as a mixture (22 mg).

Corymbotin A [**6**].—Colorless oil; $[\alpha]^{25}D$ −52.3° (*c* = 0.022, $CDCl_3$); ^1H and ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M - MeOH]^+$ 458 (0.2), $[M - HOAc]^+$ 430 (0.7), 370 (5), 356 (9), 328 (15), 296 (11), 60 (21), 43 (100); hrms calcd for $C_{26}H_{34}O_7$, 458.2304, found 458.2281.

Corymbotins B [**7**] *and C* [**8**].—The ^1H- and ^{13}C-nmr spectra were virtually identical to those of corymbotin A, with the resonances of the C-2 acetate replaced by resonances for the isobutyrate [^1H nmr δ 2.64 (septet, 1, *J* = 7.0 Hz), 1.22 (d, 3, *J* = 6.7 Hz), 1.20 (d, 3, *J* = 6.5 Hz); ^{13}C nmr 176.42 (s), 33.97 (d), 19.13 (q), 18.65 (q)] and methacrylate esters [^1H nmr δ 6.16 (d, 1, *J* = 1.4 Hz), 5.64 (d, 1, *J* = 1.4 Hz), 2.00 (s, 3); ^{13}C nmr 166.61 (s), 136.54 (s), 125.56 (t), 18.26 (q)], respectively, and the C-2 resonance shifted to 65.99 ppm for the isobutyrate and 66.67 ppm for the methacrylate. Eims *m/z* (rel. int.) $[M - HOAc]^+$ 458 (0.1), $[M - HOAc]^+$ 456 (0.1).

Corymbotin D [**9**].—Colorless oil: $[\alpha]^{25}D$ −51.41° (*c* = 0.064, $CDCl_3$); ^1H and ^{13}C nmr see Table 1; eims *m/z* (rel. int.) $[M - HOAc]^+$ 356 (2), 314 (9), 296 (8), 60 (61), 43 (100); hrms calcd for $C_{24}H_{32}O_6$, 416.2199, found 416.2197.

Corymbotins E [**10**] *and F* [**11**].—The ^1H- and ^{13}C-nmr spectra were virtually identical to those of corymbotin D, with the resonances of the C-2 acetate replaced by resonances for the isobutyrate and methacrylate esters, respectively (as in compounds **7** and **8**), and the C-2 resonance shifted to 66.05 ppm for the isobutyrate and 66.72 ppm for the methacrylate. Eims *m/z* (rel. int.) $[M - HOAc]^+$ 444 (0.2), $[M - HOAc]^+$ 442 (0.1).

Corymbotin G [**12**].—Colorless oil; $[\alpha]^{25}D$ −4.45° (*c* = 0.045, $CDCl_3$); ^1H and ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M - HOAc]^+$ 418 (0.1), 358 (0.6), 316 (7), 298 (2), 135 (22), 60 (20), 43 (100); hrms calcd for $C_{23}H_{30}O_7$, 418.1991, found 418.1981.

Corymbotins H [**13**] *and I* [**14**].—The ^1H- and ^{13}C-nmr spectra were virtually identical to those of

corymbotin G, with the resonances of the C-2 acetate replaced by resonances for the isobutyrate and methacrylate esters, respectively (as in compounds **7** and **8**), and the C-2 resonance shifted to 65.88 ppm for the isobutyrate and 66.54 ppm for the methacrylate. Eims *m/z* (rel. int.) $[M - HOAc]^+$ 446 (0.02), $[M - HOAc]^+$ 444 (0.06).

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