Cordialin A and B, Two New Triterpenes from *Cordia verbenacea* DC

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Two new dammarane-type triterpenes, cordialin A and B, isolated from *Cordia verbenacea* have been identified. They have in common a 3,19-hemiacetal group bridging ring A, and an 11α-hydroxy-group. They differ in the substitution pattern of the side-chain, the former having a 20(22)en-23-one system and a 24,25-epoxide group, while the latter bears a 20(22)-ene-24,25-diol system.

WITHIN the framework of a collaborative study of biologically active compounds from Brasilian flora, *Cordia verbenacea* DC (Boraginaceae) has been investigated. Two flavones and two new triterpenes have been isolated and identified. One of the flavones, 5,6'-dihydroxy-3,6,7,3',4'-pentamethoxyflavone has been recently reported from this plant,[1] the other was found to be identical with artemetin (5-hydroxy-3,6,7,3',4'-pentamethoxyflavone) initially isolated from *Artemisia arborescens* and *A. absinthium*, Compositae.[2]

We wish to discuss the structure elucidation of the two triterpenes named cordialin A (1a) and B (2a). The two compounds have a dammarane skeleton, with a hemiacetal group bridging ring A, a hydroxy-group at C-11, and a 20,22 double bond; they differ, however, on the oxygenation level of the side-chain.

Cordialin A (1a) $C_{30}H_{46}O_5$ has in its i.r. spectrum bands at $\nu_{max.}$ 3 400, 1 670, and 1 600 cm^{-1} for a hydroxy-group and an enone system whilst its u.v. spectrum showed absorption at $\lambda_{max.}$ 255 nm (ε 13 540) characteristic of an α,β-unsaturated carbonyl grouping. Upon acetylation, a monoacetate (1b) was formed. However, by using trichloroacetyl isocyanate (TAI) with ^1H n.m.r. monitoring (Table 1), two esterifiable OH functions were detected, which were deduced to be located at C-3 and C-11.

Cordialin B (2a), $C_{30}H_{50}O_5$, showed i.r. absorption at $\nu_{max.}$ 3 350 and 1 625 cm^{-1}; since it had no u.v. absorption maxima, no carbonyl group is present but only a double bond. On the whole, the ^1H n.m.r. spectra of both compounds are closely similar and thus may be discussed together.

The hemiacetal bridge between C-3 and C-19 confers on ring A a boat conformation in which C-3 and C-10 are oriented upwards. As a consequence, the geminal C-19 protons appear as two pairs of broadened doublets at δ 4.31 and 4.15 in (1a), and at δ 4.33 and 4.19 in (2a). The geminal coupling is of J 8.5 Hz and their widening is due to two different W type interactions existing, one between 19β-H † and 1α-H (J 2.5 Hz), and a second, between 19α-H and 5α-H (J 1.0 Hz). Similar coupling values have been observed for the 19-protons in lantanilic acid [3] and rhuslactone,[4] both having a similar hemiacetal bridge. However, the chemical shift of the 19α-H in cordialin A and B appear at lower field, δ 4.15 and 4.19 respectively, as compared to δ 3.89 for lantanilic acid and 3.7 for rhuslactone. We assign this deshielding effect to the proximity of the 11α-OH group present in the cordialins; indeed, after acetylation the 19α-H signal appears at a higher field (δ 3.67). This is also well seen following interaction with TAI to produce (1c) in which the same two 19-H signals appear at δ 4.62 and 4.49. The ^{13}C n.m.r. data (Table 2) observed for ring A in the two cordialins are in agreement with those reported for a similar hemiacetal bridge present in micrandiol D.[5] The high-field value signal observed for C-6, δ 19.4 in (1a) and 19.5 in (2a), is characteristic for the dammarane skeleton, and is the result of three γ effects due to the three methyl carbons 18, 19, and 29.[6]

The occurrence of an 11-hydroxy-group in the dammarane series is rather unusual and contrasts with the C-12 position more common for these compounds. Its equatorial orientation was deduced from the coupling constant of the adjacent 11β-H which is typical for a *trans*-diaxial interaction (J 11 Hz). Such an α-equatorial orientation of the hydroxy-group induces a strong deshielding effect on the chemical shift of the 1β-H (δ 3.12). This effect decreases in the acetate (1b) and the carbamate (1c). Supporting evidence for the presence of the hydroxy-group at C-11 can be found by careful analysis of ^{13}C n.m.r. data. Comparing the values of (1a) and its acetate (1b), one can see that the signal of the tertiary carbon which is adjacent to the hydroxylated carbon appears at δ 50.0 and 47.6 p.p.m. respectively. Now, comparing the values for cordialin A (1a) to B (2a), it can be seen that this signal at δ 50.0 p.p.m. for the former is not influenced by the different side-chain in the latter (δ 50.2). On the other hand, due to the different side-chains, values observed for the ring D carbons change from one to the other. Should a hydroxy-group be present at C-12, the adjacent tertiary carbon (then supposedly located at C-13), would exhibit different chemical shifts, which is not the case.

We have already seen that the difference between cordialin A and B resides in the side-chain, the double bond being conjugated in the former to a carbonyl group.

† 19α- and 19β-H Provide the relative orientations towards the ring formed by the hemiacetal bridge.

TABLE 1

Relevant ^{1}H n.m.r. signals of cordialins

	(1a)	(1b)	(1c)	(2a)	(2b)
1β-H	3.12 (td, 12.5, 6)	2.65 (td, 12.5, 6)	2.68 (td, 12.5, 6)	3.16 (td, 12.5, 6)	2.65 (td, 12.5, 6)
11β-H	3.65 (td, 11, 4.5)	4.78 (td, 11, 4.5)	5.56 (td, 11, 4.5)	3.59 (td, 11, 4.5)	4.80 (m)
17α-H	2.40 (td, 11, 6)	2.34 (td, 11, 6)	2.50 (td, 11, 6)	2.25 (td, 11, 6)	2.31 (td, 11, 6)
19α-H	4.15 (dd, 8.5, 1)	3.67 (dd, 8.5, 1)	4.49 (d, 8.5)	4.19 (dd, 8, 1)	3.68 (dd, 9.3, 1.5)
19β-H	4.31 (dd, 8.5, 2.5)	4.28 (dd, 8.5, 2.5)	4.62 (d, 8.5)	4.33 (dd, 8, 2.5)	4.28 (dd, 9.3, 3)
22-H	6.28 (bs, $W_{\frac{1}{2}}$ 3.7)	6.24 (bs, $W_{\frac{1}{2}}$ 4.2)	6.28 (bs, $W_{\frac{1}{2}}$ 3.7)	5.24 (bt, 7)	5.10 (t, 7.7)
24-H	3.34 (s, $W_{\frac{1}{2}}$ 2)	3.32 (s, $W_{\frac{1}{2}}$ 2)	3.34 (s, $W_{\frac{1}{2}}$ 2)	3.37 (m, $W_{\frac{1}{2}}$ 17.5)	4.80 (m)
Methyl groups					
18	0.95 (s, $W_{\frac{1}{2}}$ 2.5)	0.99 (s, $W_{\frac{1}{2}}$ 2.5)	1.02 (s, $W_{\frac{1}{2}}$ 2.5)	0.92 (s, $W_{\frac{1}{2}}$ 2.5)	0.99 (s, $W_{\frac{1}{2}}$ 2.5)
21	2.12 (bs, $W_{\frac{1}{2}}$ 2.5)	2.11 (bs, $W_{\frac{1}{2}}$ 2.5)	2.11 (bs, $W_{\frac{1}{2}}$ 2.5)	1.56 (bs, $W_{\frac{1}{2}}$ 5)	1.53 (bs, $W_{\frac{1}{2}}$ 5)
26	1.42 (s, $W_{\frac{1}{2}}$ 1.5)	1.42 (s, $W_{\frac{1}{2}}$ 1.5)	1.42 (s, $W_{\frac{1}{2}}$ 1.5)	1.16 (s, $W_{\frac{1}{2}}$ 1.5)	1.20 (s, $W_{\frac{1}{2}}$ 1.5)
27	1.27 (s, $W_{\frac{1}{2}}$ 1.5)	1.25 (s, $W_{\frac{1}{2}}$ 1.5)	1.25 (s, $W_{\frac{1}{2}}$ 1.5)	1.22 (s, $W_{\frac{1}{2}}$ 1.5)	1.20 (s, $W_{\frac{1}{2}}$ 1.5)
28	1.03 (s, $W_{\frac{1}{2}}$ 2.5)	1.04 (s, $W_{\frac{1}{2}}$ 2.5)	1.03 (s, $W_{\frac{1}{2}}$ 2.5)	1.03 (s, $W_{\frac{1}{2}}$ 2.5)	1.03 (s, $W_{\frac{1}{2}}$ 2.5)
29	1.01 (s, $W_{\frac{1}{2}}$ 2.5)	0.90 (s, $W_{\frac{1}{2}}$ 2.5)	1.14 (s, $W_{\frac{1}{2}}$ 2.5)	1.01 (s, $W_{\frac{1}{2}}$ 2.5)	0.97 (s, $W_{\frac{1}{2}}$ 25)
30	0.90 (s, $W_{\frac{1}{2}}$ 2.5)	0.93 (s, $W_{\frac{1}{2}}$ 2.5)	1.02 (s, $W_{\frac{1}{2}}$ 2.5)	0.88 (s, $W_{\frac{1}{2}}$ 2.5)	0.88 (s, $W_{\frac{1}{2}}$ 2.5)
Others					
CH_3CO		2.04 (s, $W_{\frac{1}{2}}$ 1.1)			2 × 2.03 (s)
TAI Carbamates			8.6 (s, $W_{\frac{1}{2}}$ 4.5) 9.6 (s, $W_{\frac{1}{2}}$ 4.5)		

Chemical shifts are expressed as δ values coupling constants (in Hz) are in parentheses. Abbreviations: s = singlet; bs = broad singlet; d = doublet; dd = double doublet; td = triple doublet; m = multiplet.

TABLE 2

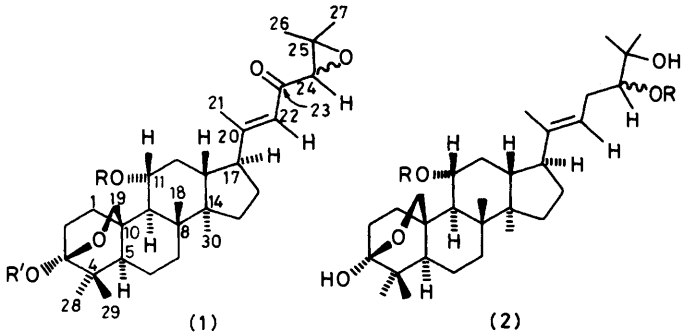
^{13}C N.m.r. data of cordialins (in δ units)

Carbon	(1a)	(1b)	(2a)	Carbon	(1a)	(1b)	(2a)
1	29.8	29.7	29.9	17	51.5	51.4	49.7
2	37.8	37.5	37.8	18	15.4	15.3	15.4
3	98.7	98.3	98.6	19	67.8	67.8	67.9
4	36.1	36.0	36.2	20	164.8	163.7	139.6
5	50.9	50.7	51.1	21	17.3	17.2	13.4
6	19.4	19.4	19.5	22	120.4	120.7	121.4
7	34.3	34.1	34.3	23	195.7	195.7	30.5
8	39.9	40.2	39.9	24	66.4	66.4	78.1
9	50.0	47.6	50.2	25	61.2	61.0	72.8
10	41.0	41.0	41.0	26	24.9	24.9	26.4
11	70.5	73.0	70.8	27	18.6	18.7	23.7
12	36.9	32.2	37.1	28	26.4	26.4	26.4
13	44.8	44.2	43.3	29	18.8	18.7	18.8
14	49.0	48.9	48.4	30	16.8	16.6	16.8
15	31.7	31.6	31.3	CH_3CO	—	21.8	—
16	27.9	27.8	27.4	CH_3CO	—	169.8	—

The ^{1}H n.m.r. evidence is as follows: the signal of the 21-Me is in (1a) at low field (δ 2.12) indicating that it is allylic, and the signal of the vinylic 22-H appears at 6.28 as a broad singlet. Sequential double irradiations showed that the 22-H exhibit only small coupling constants (less than 2 Hz) with all its neighbours: the two allylic positions 17α-H and 21-H, and the more distant epoxy-proton 24-H (δ 3.34). In cordialin B (2a), however, the olefinic 22-H gives rise to a broad triplet, δ 5.24 (*J* 6.2 Hz), due to the 23-methylene which replaces the carbonyl group in this position. In this case, double irradiation also results in changes similar to these observed for cordialin A (1a), *i.e.*, alterations in the shape of the signals for 17α-H and 21-H. The *E*-configuration of the 20,22 double bond was deduced from ^{13}C n.m.r. data, mainly by the relative high-field location of the signal related to C-21, δ 17.3 for (1a), this value is very close to that of the same carbon atom in cholest-20(22)-

ene located at δ 17.8 for the *E*-isomer, whereas the signal for the *Z*-isomer is at 22.8.[7]

The occurrence of the oxiran ring in the side-chain of cordialin A (1a) was disclosed primarily by the mass spectrum: a peak was observed at *m/z* 71 for C_4H_7O corresponding to the fragmentation shown in the Scheme,

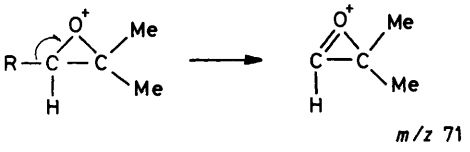


(1) (2)

a; R = R′ = H a; R = H

b; R = Ac, R′ = H b; R = Ac

c; R = R′ = TAI ester



m/z 71

SCHEME

which is characteristic for such a grouping, as described for melianone [8] and turraeanthin.[9] Also fragments were observed for the loss of 71 in (1a) *m/z* 415 (20%) and for (1b) 457 (25%). Corroboration was obtained in the ^1H n.m.r. spectrum by the location of the 24-epoxidic proton at δ 3.3. In cordialin B (2a), a diol is present at positions C-24,25 being a secondary tertiary system. The presence of the tertiary OH group at C-25 is shown by a peak *m/z* 59 assigned to a fragment $(CH_3)_2\overset{+}{C}{=}O$ already encountered in other similar cases.[10] This was supported by a peak at 413 for $M - 59 - H_2O$, and by n.m.r. signals for the two geminal 26,27-methyl groups appearing as sharp singlets at δ 1.16 and 1.22. N.m.r. considerations lead to the assignment of the adjacent 24-OH. Indeed, since the 24-H signal is unaffected during the irradiation at the signal position of the olefinic 22-H, the OH group cannot be placed at C-23. The presence of the methylene group at C-23 had been established earlier. Further evidence for the presence of the secondary 24-OH group was obtained by the formation of a diacetate of cordialin B (2b). Following this acetylation, the n.m.r. signal of 24-H shifts from δ 3.37 to 4.80 as expected, and overlaps the signal of the 11β-H (δ 4.80 for 2 × H). The two acetate groups appear as two very close signals at δ 2.03. In the mass spectrum of cordialin B diacetate (2b), again the *m/z* 59 peak appears for the tertiary OH groups, as well as a peak at 515 for $M - 59$. Also the double loss of the elements of acetic acid is observed for this compound.

One can assume that these two compounds are biogenetically interrelated, the diol being formed by the opening of the oxiran ring. A 24,25-epoxide accompanied by its corresponding diol has already been found in a number of cases, for example, in melianol and meliantriol.[11]

EXPERIMENTAL

M.p.s were taken on a Fischer-Johns apparatus. Optical rotations were recorded with an automatic Perkin-Elmer 141 polarimeter and refer to solutions in chloroform. I.r. spectra were recorded on a Perkin-Elmer 467 grating spectrophotometer and refer to KBr pellets; u.v. spectra were recorded on a Cary 118 instrument for solutions in ethanol; ^1H and ^{13}C n.m.r. spectra were determined on Brüker WH-270 and WH-90 (at 22.63 HHz) instruments respectively for chloroform solutions with tetramethylsilane as internal standard. T.l.c. was carried out on chromatoplates of E Merck (200 × 200 mm, silica gel F_{254}). Preparative chromatoplates 2 mm thickness were used (200 × 200 mm, silica gel F_{254}) for mixture separations. Mass spectra were taken under the direction of Dr. Z. Zaretskii with a Varian MAT 731 HR instrument and improved Atlas CH4 instrument. Analyses were performed in the microanalytical laboratory of our Institute by Mr. R. Heller.

Isolation.—*Cordia verbenacea* DC, Boraginaceae [12] is a shrub growing on the Brasilian Atlantic coast reputed in popular medicine to be an anti-inflamatory drug. Leaves were collected at Praia Grande, province of São Paulo, dried at room temperature, and extracted with acetone. After concentration under reduced pressure, the extract was freed from chlorophyll and waxes by refluxing with hexane. Thus, 1 kg of dried material furnished 80 g of viscous residue which was then chromatographed on silica gel columns in portions of 10 g. The hexane–ethyl acetate eluates afforded 5,6′-dihydroxy-3,6,7,3′,4′-pentamethoxyflavone and artemetin as a mixture which was resolved by repeated column chromatography, and fractional crystallisations. Further elutions with hexane–ethyl acetate (1 : 1 and 1 : 2) gave mixtures of cordialin A and cordialin B which were resolved by a number of column chromatographies and careful recrystallisations.

Artemetin.—This compound (yield 1.2%) had m.p. 168—170 °C from acetone–hexane, $\nu_{max.}$ 3 400, 1 650, 1 620, 1 460, 1 390, and 1 360 cm^{-1}; M^+ 388 (Found: C, 62.05; H, 5.15. $C_{20}H_{20}O_8$ requires C, 61.72; H, 5.18%).

5,6′-Dihydroxy-3,6,7,3′,4′-pentamethoxyflavone.—This compound (yield 1.5%) had m.p. 206—209 °C from acetone; $\nu_{max.}$ 3 400, 1 640, 1 600, 1 500, 1 450, and 1 360 cm^{-1}; M^+ 404 (Found: C, 59.1; H, 4.9. $C_{20}H_{20}O_9$ requires C, 59.41; H, 4.99%).

Cordialin A (1a) [*24,25-Epoxy-11α-hydroxydammar-20(22)-en-23-one 3,19-Hemiacetal*].—This compound (yield 0.8%) had m.p. 112—113 °C from acetone–hexane; $[\alpha]_D$ +80.7° (*c* 0.3); $\lambda_{max.}$ 255 nm (ε 13 540); $\nu_{max.}$ 3 400, 1 670, 1 600, 1 250, and 810 cm^{-1}; *m/z* 486 (M^+) (2%), 468 ($M - H_2O$) (2), 453 ($M - H_2O - 15$), (4), 450 ($M - 2 \times H_2O$)(1), 415 ($M - 71$) (20), and 71 (C_4H_7O) (18); high-resolution mass spectrum 487.3454 ($M^+ + 1$ for $C_{30}H_{46}O_5 + H$) (3), 469.3288 ($M - H_2O + H$) (8.5), and 71.0516 (18.6).

Cordialin A Acetate (1b) [24,25-*Epoxy*-11α-*acetoxydammar*-20(22)-*en*-23-*one* 3,19-*Hemiacetal*].—Cordialin A (180 mg) was acetylated by treatment with acetic–anhydride pyridine at room temperature overnight. The product was purified by chromatography [hexane–ethyl acetate (2 : 1)], and crystallised from ethanol (100 mg), m.p. 225—230 °C, $[\alpha]_D$ +34.9° (*c* 0.3); $\lambda_{max.}$ 255 nm (ε 12 670); $\nu_{max.}$ 3 450, 1 720, 1 670, 1 690, 1 235, and 800 cm^{-1}; *m/z* 528 (M^+) (5%) 468 (M − AcOH) (2), 71 (15); high-resolution mass spectrum 528.3450 (M^+ for $C_{32}H_{48}O_6$) (7.4%) 457.2956 (M − 71) (25), and 397.2720 (M − 71 − AcOH) (19) (Found: C, 72.5; H, 9.15. $C_{32}H_{48}O_6$ requires C, 72.69; H, 9.15%).

Cordialin B (2a) [*Dammar*-20(22)-*ene*-11α-24,25-*triol* 3,19-*Hemiacetal*].—This compound (yield 0.15%) had m.p. 114—115 °C from acetone; $[\alpha]_D$ +71° (*c* 0.09); $\nu_{max.}$ 3 350 and 1 625 cm^{-1}; *m/z* 490 (M^+) (4%), 475 (M − 15) (0.7), 472 (M − H_2O) (8), 454 (M − 2 × H_2O) (3), 436 (M − 3 × H_2O) (1), 413 (M − H_2O − 59) (1), and 59 [$(CH_3)_2\overset{+}{C}$=O] (38).

Cordialin B Diacetate (2b) [11α,24-*Diacetoxydammar*-20(22)-*en*-25-*ol* 3,19-*Hemiacetal*.—The acetate (2b), prepared as above, had m.p. 190—191 °C from ethyl acetate; $[\alpha]_D$ 0° (*c* 0.11); $\nu_{max.}$ 3 350, 2 910, 1 720, and 1 240 cm^{-1}; *m/z* 515 (M − 59) (3), 514 (M − AcOH) (8), 454 (M − 2 × AcOH) (6), and 59 [$(CH_3)_2\overset{+}{C}$=O] (18) (Found: C, 67.85; H, 9.4. $C_{34}H_{54}O_7 \cdot 1.5H_2O$ requires C, 67.85; H, 9.55%).

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