

## NEW TETRACYCLIC DITERPENES FROM *CROTON ARGYROPHYILLOIDES*<sup>1</sup>

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**ABSTRACT.**—The EtOH extract from *Croton argyrophyloides* trunk wood afforded a new diterpenic acid (**1**) and the corresponding methyl ester (**2**), both with a  $\delta$ -lactonic ring system. Structural determinations were made by spectroscopic data and chemical reactions.

The family Euphorbiaceae is widespread in northeastern Brazil, being represented by several species in the genus *Croton* (1,2). *Croton argyrophyloides* Muell. Arg. is a small shrub very common in the hinterland of the semi-arid regions. Previous work on the roots of this species yielded a diterpene with antibiotic activity (3), whereas the leaves produced an essential oil containing  $\alpha$ -pinene, sabinene, 1,8-cineole,  $\beta$ -elemene,  $\beta$ -caryophyllene,  $\alpha$ -humulene, and  $\gamma$ -elemene (4).

The present work deals with the isolation of a tetracyclic diterpenic acid with a six-member  $\delta$ -lactonic ring system and its methyl ester.

### RESULTS AND DISCUSSION

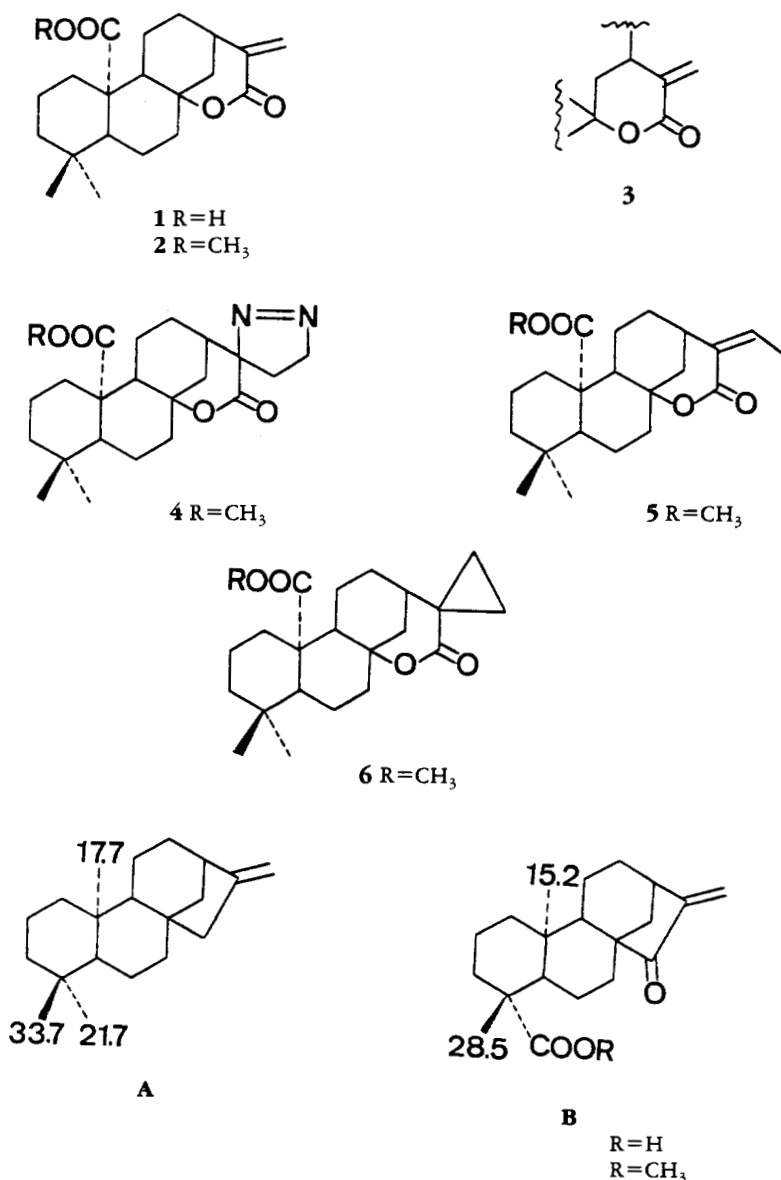
Fractionation of the EtOH extract by silica gel column chromatography afforded two crystalline substances named Aa-7CM and Aa-1CM. Uncoupled <sup>13</sup>C-nmr spectrum of Aa-7CM (mp 182-184°) showed only 20 spectral lines, but SFORD analysis of the absorption at  $\delta$  52.15 was consistent with two coalescent lines (OCH<sub>3</sub> and CH). The molecular formula C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>, inferred from the ion *m/z* 346, was confirmed by high resolution ms: M<sup>+</sup> 346.2131 (calcd for C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>, 346.2144); ir absorptions at 3060, 1714, 1635, and 815 cm<sup>-1</sup> as well as <sup>1</sup>H-nmr absorptions at  $\delta$  6.35 and 5.55 (two broad singlet 2H) and <sup>13</sup>C-nmr absorptions at  $\delta$  139.65 (s,C),  $\delta$  126.82 (t,C),  $\delta$  126.82 (t,C),  $\delta$  166.07 (s,C=O) are in agreement with an exocyclic double bond in conjugation with a carbonyl group (5-7). The presence of a carbomethoxy group was assigned by the <sup>1</sup>H-nmr sharp absorption at  $\delta$  3.62 (s,3H), <sup>13</sup>C-nmr peaks at  $\delta$  178.78 (s,C=O) and  $\delta$  52.15 (q,OCH<sub>3</sub>), and confirmed by ir absorptions at 1720, 1230, 1185, and 1160 cm<sup>-1</sup> (8-10). A <sup>13</sup>C-nmr absorption at  $\delta$  85.25 (11,12) suggested that the fourth oxygen apparent from the molecular formula is part of a  $\delta$ -lactonic residue (**3**). A molecular formula C<sub>21</sub>H<sub>30</sub>O<sub>4</sub> with three unsaturations (two carbonyl groups and one double bond) is consistent with a tetracyclic system in Aa-7CM. This was confirmed by comparison with data from similar tetracyclic diterpenes (7,8).

Further analysis of the <sup>1</sup>H-nmr spectrum allowed the assignment of gem-methyl groups ( $\delta$  1.26 and  $\delta$  1.17, s, 6H), which was confirmed by peaks at  $\delta$  34.58 (q, CH<sub>3</sub> equatorial) and  $\delta$  18.0 (q, CH<sub>3</sub> axial) in the <sup>13</sup>C-nmr spectrum. The positioning of a carbomethoxy group for C-20 was suggested by analysis of the <sup>13</sup>C-nmr spectrum and further comparison with similar diterpenic structures (10). Observing the chemical shifts of C-18, C-19, and C-20 in structures **A** and **B**, it is possible to verify that the

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presence of a  $-\text{COOCH}_3$  group in position C-19 protects  $\delta$  5.25 in the chemical shift of the C-18 methyl ( $\gamma$ -effect of the oxygen atom). The chemical shifts observed for the methyls in Aa-7CM ( $\delta$  34.58 and 18.0) ensure the presence of a  $\text{CH}_3$  group in position C-18 ( $\delta$  34.58, see **A**) and eliminates the possibility of a carbomethoxy function in C-19. In the latter case, the absorption of  $\text{CH}_3$ -18 would be expected at  $\delta$  28.50 (see **B**) and not at  $\delta$  18.0, as observed in the  $^{13}\text{C}$ -nmr spectrum of Aa-7CM. All the above facts, supplemented by calculations of  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  effects using the  $^{13}\text{C}$ -nmr chemical shift theory and SFORD analysis, allowed the proposal of structure **2** for Aa-78CM.

The hypothesis was confirmed by hydrogenation of Aa-7CM in the presence of Pd/C, giving a dihydroderivative showing no olefinic double bond by ir,  $^{13}\text{C}$ -nmr, or  $^1\text{H}$ -nmr spectral analysis. Furthermore, it was possible to assign unambiguously the  $\alpha$ ,  $\beta$ -unsaturated  $\delta$ -lactonic system in Aa-7CM by 1-pyrazoline formation using the well-



known reaction of  $\text{CH}_2\text{N}_2$  with a conjugated carbonyl system (13-17). Indeed, when this diterpene was allowed to react with  $\text{CH}_2\text{N}_2$  at  $0^\circ$ , the product obtained was **4**, showing absorptions at  $1560\text{ cm}^{-1}$  ( $\nu\text{ N}=\text{N}$ ) in the ir spectrum and a broad band in the  $^1\text{H}$ -nmr spectrum ( $\delta$  4.5-5.0, 2H) assigned to  $\text{CH}_2$  bonded to  $\text{N}=\text{N}$ . In addition, pyrolysis of **4** at  $140^\circ$  produced the expected mixture of **5** and **6** (13, 18, 19). The presence of a cyclopropane ring in the mixture was inferred from absorption in the region 0.64-1.35  $\delta$  in the  $^1\text{H}$ -nmr spectrum. A quartet centered at  $\delta$  6.28 ( $J=7.4\text{ Hz}$ , 1H) and a doublet at  $\delta$  2.20 ( $J=7.4\text{ Hz}$ , 3H) are in agreement with the system  $\text{CH}_3\text{CH}=\text{C}$ . This was confirmed by a series of  $^1\text{H}$ -nmr double irradiation experiments.

The second substance, named Aa-1CM, gave white crystals, mp  $212\text{-}214^\circ$ , and showed similar spectral data to Aa-7CM. The absence of methoxy group absorption in the  $^1\text{H}$ -nmr spectrum and the presence of an absorption at  $1680\text{ cm}^{-1}$  in the ir spectrum characteristic of a carboxylic acid allowed the assignment of structure **1** for Aa-1CM, the corresponding acid for Aa-7CM. The reaction of **1** with  $\text{CH}_2\text{N}_2$  produced the pyrazoline (**4**).

## EXPERIMENTAL

**ISOLATION OF CONSTITUENTS.**—A wood sample of *C. argyrophylloides* (voucher 3088, Herbarium, Departamento de Biologia, da Universidade Federal do Ceará), was reduced to powder (16.2 kg) and percolated with EtOH. The extract (3.6 kg) was adsorbed on silica gel and chromatographed. Elution with hexane, hexane- $\text{CHCl}_3$  (1:1) and  $\text{CHCl}_3$ -MeOH (99:1) gave two fractions, Aa-1CM and Aa-7CM.

**Aa-7CM (2).**—Aa-7CM (180 mg) crystallized from hexane-Et<sub>2</sub>O, mp  $182\text{-}184^\circ$ ; Found:  $\text{M}^+$  346.2131.  $\text{C}_{21}\text{H}_{30}\text{O}_4$  requires  $\text{M}^+$  346.214; ir  $\nu$  max (KBr) 3060, 2970, 1720, 1714, 1635, 1300, 1230, 1195, 1160, 1020, 950,  $815\text{ cm}^{-1}$ ; ms  $m/z$  (%) 346 ( $\text{M}^+$ , 20), 331 (56), 288 (18), 287 (100), 286 (78), 271 (41), 231 (7), 227 (18);  $^{13}\text{C}$ -nmr (25.2 MHz,  $\text{CDCl}_3$ )  $\delta$  36.74 (t, C-1), 18.27 (t, C-2), 42.01 (t, C-3), 39.11 (s, C-4), 52.53 (d, C-5), 22.13 (t, C-6), 40.07 (t, C-7), 85.25 (s, C-8), 49.65 (d, C-9), 47.57 (s, C-10), 16.33 (t, C-11), 30.91 (t, C-12), 52.15 (d, C-13), 34.58 (t, C-14), 166.07 (s, C-15), 139.05 (s, C-16), 126.82 (t, C-17), 18.00 (q, C-18), 34.58 (q, C-19), 178.78 (s, C-20), 52.15 (q,  $\text{OCH}_3$ );  $^1\text{H}$ -nmr (100 MHz,  $\text{CDCl}_3$ )  $\delta$  3.62 (s, 3H,  $\text{OCH}_3$ ), 2.34 (dd, 1H,  $J=14.5$  and  $J=4.0\text{ Hz}$ , H-14 eq) 1.9-1.42 (broad band), 1.26 (s, 3H,  $\text{CH}_3$ ), 1.17 (s, 3H,  $\text{CH}_3$ ), 6.35 (sb, 1H), 5.55 (sb, 1H). Aa-7CM (18 mg) in  $\text{CH}_3\text{OH}$  (3.5 ml) was hydrogenated over 10% Pd/C (12 mg), filtered, and evaporated. Dihydro-Aa-7CM (15 mg) was purified by crystallization from hexane- $\text{CH}_3\text{OH}$ , mp  $155\text{-}158^\circ$ ; ir  $\nu$  max (KBr) 2960, 2940, 1718 (broad band), 1460, 1380, 1355, 1225, 1140, 1110, 980, 880, 790,  $760\text{ cm}^{-1}$ ;  $^1\text{H}$ -nmr (100 MHz,  $\text{CDCl}_3$ ) identical to spectrum for Aa-7CM with the exception that signals from olefinic protons at  $\delta$  5.55 and 6.35 disappeared; ms ( $m/z$ ) 348 ( $\text{M}^+$ , 5%).

**Pyrazoline of Aa-7CM (4).**—Aa-7CM (30 mg) in Et<sub>2</sub>O (3.0 ml) was allowed to react with excess  $\text{CH}_2\text{N}_2$ . The residue was extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  was dried and evaporated under vacuum giving a crystalline residue (20 mg), mp  $137\text{-}140^\circ$ ; ir  $\nu$  max (KBr) 2980, 2945, 1720, 1560, 1460, 1360, 1305, 1223, 1105, 1065,  $890\text{ cm}^{-1}$ ; uv  $\lambda$  max (MeOH) nm (log  $\epsilon$ ): 217 (4.84) and 330 (4.19);  $^1\text{H}$ -nmr (60 MHz,  $\text{CDCl}_3$ )  $\delta$  4.5-5.0 (m, 2H,  $\text{CH}_2\text{-N}=\text{N}$ ), 3.62 (s, 3H,  $\text{OCH}_3$ ), 2.86 (m, 2H,  $\text{CH}_2\text{-CH}_2\text{-N}=\text{N}$ ), 2.32 (dd,  $J=14.5\text{ Hz}$  and  $J=4.0\text{ Hz}$ , 1H eq-14), 1.45-2.06 (broad band), 1.15 (s, 3H,  $\text{H}_3\text{C-18}$ ), 1.06 (s, 3H,  $\text{H}_3\text{C-19}$ ).

**Thermolysis of Aa-7CM pyrazoline (4).**—Pyrazoline (12 mg) in xylene (2.0 ml) was heated in an oil bath ( $140^\circ$ ) with stirring for 0.5 h. The solvent was removed under vacuum, giving an amorphous residue that recrystallized in hexane-MeOH as a mixture of two substances.  $^1\text{H}$ -nmr (60 MHz,  $\text{CDCl}_3$ )  $\delta$  6.28 (q,  $J=7.4\text{ Hz}$ , 1H,  $\text{H}_3\text{C-CH}=\text{C}$ ), 3.7 (s, 3H,  $\text{OCH}_3$ ), 2.4-3.0 (m), 2.2 (d,  $J=7.4\text{ Hz}$ , 3H,  $\text{H}_3\text{CCH}=\text{C}$ ), 1.4-2.1 (m), 1.2 (s, 3H,  $\text{H}_3\text{C-18}$ ), 1.1 (s, 3H,  $\text{H}_3\text{C-19}$ ), 0.64-1.35 (m, cyclopropane).

**Pyrazoline of Aa-1CM (4).**—Reaction of **1** with  $\text{CH}_2\text{N}_2$  produced the pyrazoline (**4**).

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