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NOR-CLERODANE DITERPENES FROM *CROTON CAJUCARA*

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Key Word Index—*Croton cajucara*; Euphorbiaceae; Sacaca, *nor*-clerodane diterpene; *t*-crotonin; dehydrocrotonin; *t*-dehydrocrotonin

Abstract—A new *nor*-clerodane diterpene, *t*-crotonin, was isolated from the cortices of *Croton cajucara* along with the previously isolated diterpene, of which the structure was not confirmed, dehydrocrotonin. The structures of these compounds were elucidated by chemical and spectroscopic methods.

INTRODUCTION

Croton cajucara Benth is a Brazilian medicinal plant (commonly called 'Sacaca'), its cortices are known for their antidiabetic and antilipotropic properties [1]. From the dichloromethane extract of cortices, two *nor*-clerodane diterpenes were isolated. In this paper, we report the isolation and structural elucidation of a new diterpene, *t*-crotonin, and the previously isolated diterpene, dehydrocrotonin. The latter compound has not previously had its structure confirmed conclusively.

RESULTS AND DISCUSSION

The cortices of *C. cajucara* purchased in Belém, Brazil, were extracted with dichloromethane. Silica gel column chromatography and subsequent HPLC of the extract led to the isolation of *t*-crotonin (**1**) and dehydrocrotonin (**2**).

t-Crotonin, $C_{19}H_{24}O_4$, IR spectrum showed γ-lactone carbonyl (1770 cm^{-1}), six-membered ring carbonyl (1720 cm^{-1}) and furanic (1510, 875 cm^{-1}) absorptions. The ^1H NMR showed characteristic signals for a β-substituted furan ring (δ6.37, 7.43, 7.43) and two secondary methyl groups (δ1.05, 1.14). In the mass spectrum, the fragmentation of the molecular ion (*m/z* 316) gave the base peak at *m/z* 94. Other major fragmentation peaks were observed at *m/z* 222, 207, 177 and 81. These spectral data were similar to those of crotonin (**5**), which was isolated from *Croton lucidus* [2–4]. However, the assignment of its ^1H NMR spectrum (Table 1) by means of the ^1H–^1H COSY and decoupling experiments, was different from that of crotonin. This difference suggested that **1** was stereoisomer of crotonin.

The coupling constant between H-5 and H-10 (J = 10.7 Hz) indicated a *trans* A/B ring junction instead of *cis* for crotonin; so **1** was revealed to be of the *trans* clerodane type. Other parts of the relative stereochemistry of **1** were clarified on the basis of the coupling constants of each proton and the NOESY spectrum.

A methyl group at C-4 was assigned as an equatorial one and a proton at C-4 assigned as an axial one since the J value between H-4 and H-5 was 10.7 Hz The methyl group at C-8 was deduced to be equatorial, because the proton at C-8 was axial from its coupling constants with protons at C-7 (J = 13.1 and 3.4 Hz). The stereochemistry at C-9 of the clerodane type diterpene was supported by the presence of NOESY cross peaks among H-11, H-8 and H-10. In the NOESY spectrum, the strong cross peak between Me-17 and H-12 showed the C-12 stereochemistry as shown in **1**.

To determine the absolute configuration of **1**, the octant rule was applied. The CD spectrum of **1** showed a positive Cotton effect ($\Delta\varepsilon_{285} = +0.19$), so the absolute configuration was determined to be as shown in Fig 1. Thus the absolute structure of **1** was (12*R*)-15,16-epoxy-19-*nor*-*neo*-cleroda-13(16),14-diene-20,12-olide-2-one.

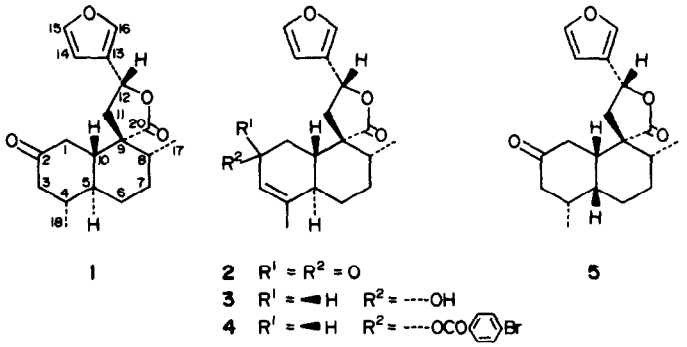

Fig. 1.

Table 1 ^{1}H NMR data of compounds **1**, **2** and **3** ($CDCl_3$, TMS as int standard)*

	1	2	3
H-1α	2 15†	2 18 *dd*	1 24 *ddd*
H-1β	2 48 *ddd*	2 54 *dd*	2 13 *ddd*
H-2	—	—	4 19 *m*
H-3	2 15†	5.89 *dd*	5 37 *br s*
H-3β	2 33 *ddd*	—	—
H-4	1 42 *m*	—	—
H-5	2.04 *dddd*	3 18 *m*	2 81 *m*
H-6α	2 15†	2 26 *dddd*	2 10 *dddd*
H-6β	0.94 *dddd*	1 18 *dddd*	0 96 *dddd*
H-7α	1 80 *dddd*	1 88 *dddd*	1 77 *dddd*
H-7β	1 57 *dddd*	1 59–1 74†	1 56–1.74†
H-8	1 66 *m*	1 59–1.74†	1.56–1 74†
H-10	1.47 *ddd*	1 80 *ddd*	1 32 *ddd*
H-11	2 31 *dd*	2.36 *dd*	2 40 *dd*
H-11	2 42 *dd*	2.43 *dd*	2 42 *dd*
H-12	5 40 *dd*	5 43 *dd*	5 41 *dd*
H-14	6.37 *dd*	6 41 *dd*	6.43 *dd*
H-15	7 43†	7.45†	7 44 *dd*
H-16	7.43†	7.46†	7 46 *dd*
Me-17	1 14 *d*	1.16 *d*	1 12 *d*
Me-18	1 05 *d*	1 97 *dd*	1 69 *d*
J(Hz)			
1α,1β	13.0	15 8	12 4
1α,2	—	—	9 7
1β,2	—	—	5 9
1α,10	13 7	14 0	11 1
1β,10	2.6	2 8	4.0
1β,3β	2.6	—	—
3α,3β	14.0	—	—
3α,4	13.0	—	—
3β,4	4.2	—	—
3,18	—	1.2	1.5
3,5	—	1 2	—
4,18	6 5	—	—
4,5	10.7	—	—
5,10	10 7	10 4	10 0
5,6α	3 8	3 6	3.6
5,6β	10 7	12 8	12 7
5,18	—	1 2	—
6α,7α	3 4	3.2	3 6
6α,7β	3 4	3 6	3 6
6β,7α	13.0	12 8	12 7
6β,7β	3.4	3 6	3 4
7α,8	13 1	12.8	12 9
7β,8	3 4	†	†
8,17	6 7	6.6	6 5
11,11	14.0	14 0	15 2
11,12	8 5	8.6	8 6
11,12	8.5	8 6	8.6
14,15	1 5	1 7	1 8
14,16	0.9	0 9	0 8
15,16	†	†	1 8

*All assignments were confirmed by the ^{1}H–^{1}H COSY and decoupling experiments
†Overlapped signal

Dehydrocrotonin (**2**), another diterpene found in *C. cajucara*, had a molecular formula $C_{19}H_{22}O_4$. Its spectral data was similar to those of *t*-crotonin In the ^{1}H NMR spectrum, however, an olefinic proton appeared at δ5.89 (H-3), and a vinyl methyl group appeared at δ1.97 with long range couplings with H-3 (J = 1 2 Hz) and H-5 (J = 1.2 Hz), respectively. ^{13}C NMR also indicated the presence of an olefinic moiety (δ126.27, 165.51 ppm). Furthermore, its IR spectrum showed α,β-unsaturated ketone (C=O, 1670 cm^{-1} and C=C, 1620 cm^{-1}) absorptions. Consequently, **2** was revealed to be a 3,4-didehydro type of **1**

The absolute configuration of **2** was determined by the use of the exciton chirality method [5] on its derivative **4**. Sodium borohydride reduction of **2** afforded **3** which possessed an allyl alcohol moiety in its molecule A hydroxy group at C-2 was equatorial, because a proton at C-2 (δ4.19) was an axial one, which was clarified from its coupling constants with protons at C-1 (J = 9.7 and 5.7 Hz), and 9.0% NOE enhancement with an axial proton at C-10. **4** was formed from esterification of **3** with *p*-bromobenzoyl chloride in pyridine. The CD spectrum of **4** showed the positive Cotton effect at 242 nm ($\Delta\varepsilon$ = +11.1 in MeOH), so the asymmetric center at C-2 of **4** was established to be in the *R* configuration Consequently, the absolute structure of **2** was (12*R*)-15,16-epoxy-19-*nor*-*neo*-cleroda-3,13(16),14-triene-20,12-olide-2-one as shown.

The spectral data of **2** was identical to those of dehydrocrotonin which was isolated from *C. cajucara* by Simões *et al.* [6]. However, the structure presented by them was only tentative, and they did not discuss the absolute configuration So, this is the first time that the absolute configuration of **2** has been described.

Simões *et al.* named **2** dehydrocrotonin, which correlated with the common name crotonin, that has a *cis* A/B ring junction. We isolated *t*-crotonin from *C cajucara*,

Table 2 ^{13}C NMR data of compounds **1** and **2** ($CDCl_3$, TMS as int. standard)*

C	1	2
1	43 19	39 39
2	209.21	197.00
3	49.64	126 27
4	38 43	165 51
5	41 54	39 19
6	29 32	27 76
7	29 58	29 76
8	42.02	41 16
9	52 21	51 03
10	48 27	45 55
11	40.81	40.03
12	72.16	71 89
13	125.11	124 83
14	107 95	107 74
15	144 10	143.82
16	139 22	139 04
17	17.59	17 20
18	19 53	21 54
20	176 86	176 63

*All assignments were confirmed by the ^{13}C–^{1}H COSY experiment.

and so the name dehydrocrotonin is not suitable for compound **2** that exhibits a *trans* A/B ring junction. Accordingly, we propose the common name *t*-dehydrocrotonin for **2**, which clearly indicates a *trans*-clerodane structure.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz): $CDCl_3$ with TMS as int. standard. CC: silica gel (Kieselgel 60) at amounts equivalent to 50–100 times the sample amount, prep. HPLC: glass 22 mm i.d. × 100 mm CIG column (Kusano Scientific Co., Tokyo) packed with CPS-000-1 (10 μm silica gel) or glass 22 mm i.d. × 300 mm CIG column CPS-000-20 (20 μm ODS); TLC: 0.25 mm silica gel (60 F_{254}, Merck) or RP-18 plates (F_{254s}, Merck). Spots were detected by UV light (254 nm) and spraying with 10% H_2SO_4 and heating.

Plant material. The cortices of *Croton cajucara* were purchased in Belém, Brazil, in August 1987. The material was identified as *C. cajucara* by Dr M. Satake (Botanical garden Director, Tsukuba Medicinal Plant Research Station, National Institute of Hygienic Science, Japan).

Extraction and isolation. Dried and finely powdered cortices of *C. cajucara* were extracted with CH_2Cl_2. The extract (60 g) was chromatographed on a silica gel column and eluted with *n*-hexane–EtOAc (9:1, 4:1 and 1:1) and MeOH. This fractionation gave fractions 1–12 combined based upon TLC monitoring. Fraction 9 contained *t*-crotonin (**1**, 300 mg) which was further purified by HPLC (*n*-hexane–EtOAc–MeCN, 7:2:1 and C_6H_6–EtOAc, 23:2). Fraction 11 was subjected to silica gel CC and eluted with CH_2Cl_2–MeOH of increasing polarity. The fraction eluted with CH_2Cl_2–MeOH (99:1 and 99:2) was subjected to HPLC using *n*-hexane–EtOAc–MeCN (13:5:2) and CH_2Cl_2–MeOH (99:1). These procedures led to the isolation of compound **2** (2 g).

Compound **1** Colourless needles, mp 131–132°, $[\alpha]_D$ +2.3° ($CHCl_3$; *c* 3.29); EIMS *m/z* (rel. int.): 316 $[M]^+$ (45), 270 (5), 222 (29), 207 (8), 204 (30), 177 (8), 164 (29), 94 (100), 81 (33); HRMS: Calc. 316.1673. Found 316.1685; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2970, 1770 (C=O), 1720 (C=O), 1510, 875 (furan); UV λ_{max}^{MeOH} nm (ε): 216.0 (3000); CD: λ_{max}^{MeOH} nm (Δε): 285 (+0.19).

Compound **2**. Colourless needles, mp 138.5–140.5°, $[\alpha]_D$ +11.9 ($CHCl_3$; *c* 0.30); EIMS *m/z* (rel. int.): 314 $[M]^+$ (25), 220 (3), 161 (100), 95 (19), 94 (9), 81 (10); HRMS; Calc. 314.1516 Found 314.1504; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2970, 1760 (C=O), 1660 (C=O), 1620 (C=C), 1510, 875 (furan); UV λ_{max}^{MeOH} nm (ε). 239.0 (10200).

Reduction of compound **2**. A MeOH soln of **2** (30 mg) was treated with excess of $NaBH_4$. After work-up in the usual way, the product was purified by HPLC (*n*-hexane–EtOAc–MeCN, 7.2.1) and afforded compound **3** as a white powder, mp 118.5–120°, $[\alpha]_D$ +34.4 ($CHCl_3$, *c* 0.29), EIMS *m/z* (rel. int.): $[M]^+$ absent, 298 $[M-H_2O]^+$ (14), 204 (30), 179 (34), 145 (42), 105 (100), 95 (54), 81 (21); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 2960, 1760 (C=O), 1510, 875 (furan); UV λ_{max}^{MeOH} nm (ε): 213.5 (4400).

Preparation of **4** from **3**. Esterification of **3** with *p*-bromobenzoyl chloride in pyridine (30 min) and work-up in the usual way afforded **4** as colourless needles, mp 169.5–171.5°, $[\alpha]_D$ +85.9 ($CHCl_3$, *c* 0.14); EIMS: *m/z* (rel int.): 500 $[M]^+$ (6), 298 (25), 204 (41), 179 (30), 145 (40), 105 (100); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2970, 1760 (C=O), 1710 (C=O), 1510, 875; UV λ_{max}^{MeOH} nm (ε) 208 0 (15000), 243.5 (19300); CD λ_{max}^{MeOH} nm (Δε). 242 (+11.1); ^{1}H NMR, $CDCl_3$. 1.03 (1H, *dddd*, *J* = 12.8, 12 8, 12.8 and 3.4 Hz), 1.14 (3H, *d*, *J* = 6.5 Hz), 1.43–1.59 (2H, *m*), 1.60–1 74 (2H, *m*), 1.73 (3H, *d*, *J* = 1.3 Hz), 1.80 (1H, *dddd*, *J* = 12.2, 12.2, 12.2 and 3.3 Hz), 2.14 (1H, *dddd*, *J* = 12.8, 3.3, 3.3 and 3.3 Hz), 2.29 (1H, *dd*, *J* = 11.2 and 6.4 Hz), 2.46 (2H, *d*, *J* = 8.9 Hz), 2.91 (1H, *m*), 5.40 (1H, *d*, *J* = 1 3 Hz), 5.41 (1H, *dd*, *J* = 8.9 and 8.9 Hz), 5.55 (1H, *m*), 6.40 (1H, *dd*, *J* = 1.5 and 0.7), 7.41 (1H, *d*, *J* = 1.5 Hz), 7.46 (1H, *d*, *J* = 0.7 Hz), 7.57 (2H, *d*, *J* = 8.4 Hz), 7.88 (2H, *d*, *J* = 8.4 Hz).

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