Pergamon 0031-9422(94)E0158-O Phytochemistry, Vol. 36, No. 6, pp 1457-1463, 1994

0031 9422/94 $7.00 + 0.00

6,7-OXYGENATED NEO-CLERODANE FURAN DITERPENES FROM *CROTON SONDERIANUS*

EDILBERTO R. SILVEIRA* and JAMES D. MCCHESNEY†

Curso de Pós-Graduação em Química Orgânica, Laboratório de Produtos Naturais, Departamento de Química Orgânica e Inorgânica, Centro de Ciências, Universidade Federal do Ceará, Cx. Postal 12.200, Fortaleza-CE, 60.021, Brazil; †Research Institute of Pharmaceutical Science, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, MS 38677, U.S.A.

(*Received* 5 *November* 1993)

Key Word Index—*Croton sonderianus*; Euphorbiaceae; roots; neo-clerodane furan diterpene; 6α-hydroxyannonene; 6α,7β-dihydroxyannonene; 6α,7β-diacetoxyannonene.

Abstract—6α-Hydroxyannonene, 6α,7β-dihydroxyannonene and 6α,7β-diacetoxyannonene, three new neo-clerodane furan diterpenes, have been isolated from the hexane extractives of *Croton sonderianus* roots. Structure elucidation of these new secondary metabolites was accomplished by means of chemical derivatization and extensive spectrometric analysis, including 2D NMR experiments such as ^{1}H, ^{1}H and ^{1}H, ^{13}C-COSY.

INTRODUCTION

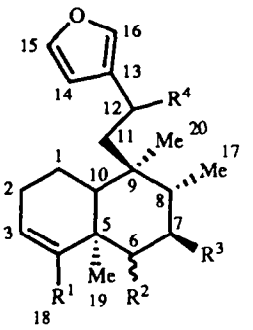
Croton sonderianus Muell. Arg., popularly known in the Brazilian Northeast as 'marmeleiro preto' is one of the most widespread shrubs on the 'caatinga', the characteristic flora of the dry region of northeast of Brazil. Used in folk medicine as a remedy for gastric disturbances it has been a very rich source of terpenoids. From the acidic portion of the hexane extractives from roots of *C. sonderianus* several diterpenes, possessing antimicrobial activity, have been isolated [1, 2]. A preliminary study of the neutral part has afforded a sesquiterpene and two neo-clerodane diterpenes [3]. Continuing our work on the more polar fractions of the extract we now report the isolation and characterization of 6α-hydroxyannonene (**1**), 6α,7β-dihydroxyannonene [**4**], and 6α,7β-diacetoxyannonene (**5**). Structure determination of these natural secondary metabolites was accomplished by chemical derivatization and interconversion, and spectrometric analysis.

RESULTS AND DISCUSSION

Silica gel chromatography of the combined fractions 18–22, using a mixture of hexane–chloroform (1:1) as eluent, allowed the isolation of a clear yellowish resin, **1**, homogeneous by TLC. Compound **1** had $[\alpha]_D$ −47.7° ($CHCl_3$; *c* 12.4) and showed a molecular ion with *m/z* 302 ($C_{20}H_{30}O_2$) and IR absorptions at 3420 cm^{-1} (OH), 1500 and 880 cm^{-1} (furan ring), and 1030 cm^{-1} (C–OH). Its ^{1}H NMR spectrum (Table 1) showed three protons corresponding to a β-substituted furan ring at δ7.40, 7.28 and 6.30 (1H each, H-15, H-16 and H-14), a vinyl proton at 5.30 (1H, *br s*, H-3), a double doublet centred at 3.60 (1H, $J = 6.0$ and 9.0 Hz, H-6), a vinyl methyl at 1.90 (H-18), two quaternary methyls, as singlets, at δ1.05 and 0.75 (H-19 and H-20), and a tertiary methyl, as a doublet, at 0.85 ($J = 6.0$ Hz, H-17). Its ^{13}C NMR decoupled spectrum (Table 2) showed 20 lines. DEPT analysis using a nutation angle of 90°, indicated four sp^2 methine carbons at δ142.8, 138.3, 122.0 and 110.9, and three saturated methines at δ75.5, 45.5 and 34.6. Besides the methine carbons, the DEPT 135° spectrum showed five methylene and four methyl carbons (see Table 2) indicating that the carbons at δ143.8, 125.5, 44.0 and 38.4 were non-hydrogenated, after comparison with the decoupled spectrum.

Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **1** with those presented by annonnene, neocleroda-3,13(16),14-triene-15,16-oxide (**2**) [3, 4], revealed that **1** was a hydroxylated derivative of annonene. The methine carbon at δ75.5 in the ^{13}C NMR spectrum was indicative of the secondary alcohol character of **1**, which in conjunction with the splitting pattern of the carbinolic proton (*dd*) suggested three possible sites for hydroxylation: C-6, C-11 or C-12. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those for 12-hydroxyhardwickic acid methyl ester (**3**) (Table 2), isolated earlier from *C. sonderianus* (**5**) eliminates C-12, an allylic position, leaving C-6 and C-11 as the only possible sites. Meanwhile, the presence of the CH_2 around δ18.0, characteristic of non-oxygenated C-12, is evidence that C-11 is also not oxygenated, because if it were, C-12 would experience the β-effect of the vicinal oxygen and should be dishielded. This leaves C-6 as the site of the hydroxyl substituent.

*Author to whom correspondence should be addressed.



	R^1	R^2	R^3	R^4
1	Me	αOH	H	H
2	Me	H	H	H
3	CO_2Me	H	H	OH
1a	Me	αOAc	H	H
1b	Me	=O	H	H
1c	Me	βOH	H	H
4	Me	αOH	OH	H
4a	Me	αOH	OAc	H
4b	Me	=O	OAc	H
4c	Me	βOH	OAc	H
5	Me	αOAc	OAc	H

In studies of cyclohexyl derivatives, by Roberts *et al.* [6], it was determined that substitution of a hydrogen with an equatorial hydroxyl group changes the α-carbon (the one that the hydroxyl is attached to) resonance downfield by 41–43.2 ppm relative to the corresponding carbon in the original, unsubstituted hydrocarbon. On the other hand, the corresponding figure for an axial hydroxyl substituent is about 35–39 ppm. The observed value for **1**, compared to hydrocarbon **2**, was 39.1 ppm (see Table 3), from which one cannot conclude with certainty anything about the stereochemistry of the hydroxyl. Meanwhile, in the same study, the researchers pointed out that the steric influences at the carbons further from the point of substitution may be more useful to assign the stereochemistry. The γ-carbon, due to 1,3 interactions, will show a larger change (6–7 ppm upfield) relative to the corresponding carbon in the hydrocarbon for the axial hydroxyl substituent than for the equatorial hydroxyl (1–1.5 ppm upfield). Due to inductive effects, as well as steric, the β-carbon undergoes a dishielding effect of the order of 5–8 ppm in both axial or equatorial cases. Comparison of the carbon resonances of **1** and **2** (Table 3) showed a large dishielding effect on the β-carbons C-5 ($\Delta\delta = 5.7$ ppm) and C-7 ($\Delta\delta = 10$ ppm), and a smaller effect on the γ-carbons C-8 ($\Delta\delta = 2.4$ ppm) and C-10 ($\Delta\delta = 1.0$ ppm). These observations suggested the assignment of the hydroxyl stereochemistry as equatorial.

Usual acetylation of **1**, in acetic anhydride–pyridine, yielded the expected monoacetate derivative (**1a**), $[M]^+$ at *m/z* 344 ($C_{22}H_{32}O_3$), whose ^1H NMR spectrum, compared to that of **1**, showed only two major differences (see Table 1): the vinyl methyl appeared at δ 1.62 ($\Delta\delta$0.3 ppm upfield) and the carbinolic methine 4.78 as a clear double doublet ($\Delta\delta$ 1.20 ppm downfield) with *J* values of 5.0 and 11.0 Hz. These observations confirmed C-6 as the oxidation site and again suggested that the carbinolic proton (H-6) couples only to two other hydrogens with an axial–axial relationship with one of them. Thus the C-6 hydroxyl must be equatorial. In order to confirm these findings we carried out the oxidation of **1** with pyridinium chlorochromate (PCC) to obtain the expected ketone (**1b**), showing $[M]^+$ at *m/z* 300 ($C_{20}H_{28}O_2$). Its ^1H NMR spectrum (Table 1) showed no absorptions lower than δ3.5 other than those expected for the furan and vinyl protons. The vinyl methyl remained at δ 1.90, one of the quaternary methyls was not affected, δ 1.0 (C-20) but the other one was strongly deshielded appearing at δ 1.45 (C-19).

Another line of evidence in support of the assigned structure came from similar comparisons with those done with **1** and **2**. A ^{13}C NMR study of cyclohexanones compared to their corresponding hydrocarbons [7] demonstrated that the effect of the carbonyl on the α-carbons (adjacent to the carbonyl) of cyclohexanones seems analogous to, but quite a bit larger than (shifted 11–18 ppm downfield), the corresponding equatorial hydroxyl effect on the β-carbons. Indeed, comparison of the ^{13}C NMR spectra of ketone **1b** and hydrocarbon **2** (Table 3) was in complete agreement with the assignment of structure **1** as the 6α-hydroxyannonene.

Finally, in order to place the analysis of the spectral data discussed above on firmer footing, we decided to examine the β-epimer of **1**. $NaBH_4$ reduction of **1b**, yielded the β-isomer (**1c**), as the major product. This was expected based on the 'steric approach control' [8] leading to the axial alcohol **1c**. This isomer showed similar IR absorptions (see Experimental) to those of **1**, and its fragmentation pattern in the mass spectrometer was similar to **1**, except for the relative intensity of some fragments, e.g. *m/z* 287 $[M-15]^+$ with relative intensity of 1.0% in **1** becomes 4.15% in **1c**; for *m/z* 284 $[M-H_2O]^+$, the rel. int. 0.1% in **1** becomes 4.77% in **1c**; and for *m/z* 269 $[M-15-H_2O]^+$, the rel. int. 0.35% in **1** becomes 28.0% in **1c**. This fragmentation behaviour is in agreement with the change in stereochemistry of the alcohol since the dehydration process taking place directly from $[M]^+$ should be favoured in isomer **1c**, where there is a hydrogen *trans*-axial to the hydroxyl group compared to **1**, in which no such arrangement is present (see Scheme 1). The loss of a methyl (C-19) *trans* to the hydroxyl is favoured in **1c** and also explains the higher relative intensity (28%) of the peak *m/z* 269 arising from the loss of water from the fragment *m/z* 287. The ^1H NMR spectrum of **1c** was also in agreement with the stereochemical changes (see Table 1), particularly for a triplet-like absorption at δ3.87 (1H, $J = 3.0$ Hz, H-6). Both the

Table 1. ^{1}H NMR spectral data for compounds **1**–**1c**, **4a**–**4e**, and **5** (δ, multiplicity, J Hz, in $CDCl_3$)

	1	1a	1b	1c	4a	4b	5	4c	4d	4e
3	5.30 *br s*	5.30 *br s*	5.47 *br s*	5.45 *br s*	5.19 *br s*	5.39 *br s*	5.99 *br s*	5.33 *br s*	5.74 *br s*	5.70 *br s*
6	3.60 *dd* (7.0, 11.0)	4.78 *dd* (5.0, 11.0)	—	3.87 *t* (3.5)	3.40 *d* (10.4)	—	5.20	3.83 *d* (3.0)	9.33 *s*	8.92 *br s*
7					4.90 *dd* (10.4, 11.2)	5.36 *d* (12.0)	4.90 2H, *m*	5.09 *dd* (3.0, 11.0)	9.90 *d* (3.0)	6.26 *br s*
14	6.30 *m*	6.33 *br s*	6.33 *br s*	6.25 *br s*	6.22 *m*	6.21 *m*	6.23 *br s*	6.26 *m*	6.26 *m*	6.36 *m*
15	7.40 *m*	7.43 *br s*	7.45 *m*	7.38 *br s*	7.36 *m*	7.32 *m*	7.40 *m*	7.36 *m*	7.36 *m*	7.36 *m*
16	7.28 *br s*	7.30 *br s*	7.30 *br s*	7.25 *br s*	7.22 *m*	7.19 *s*	7.23 *br s*	7.23 *m*	7.23 *s*	—
17	0.85 *d* (6.0)	0.85 *d* (7.0)	0.95 *d* (7.0)	0.83 *d* (7.0)	0.86 *d* (6.5)	1.05 *d* (6.5)	0.85 *d* (6.0)	0.83 *d* (7.0)	1.13 *d* (7.0)	1.80 *br s*
18	1.87 *br s*	1.62 *br s*	1.90 *br s*	1.75 *br s*	1.83 *br s*	1.85 *br s*	1.56 *br s*	1.70 *br s*	1.46 *br s*	1.43 *br s*
19	1.05 *s*	1.05 *s*	1.45 *s*	1.05 *s*	1.13 *s*	1.53 *s*	1.25 *s*	1.13 *s*	1.33 *s*	1.23 *s*
20	0.75 *s*	0.77 *s*	1.00 *s*	0.75 *s*	0.88 *s*	1.08 *s*	0.90 *s*	0.86 *s*	0.86 *s*	1.13 *s*
MeCO		2.05 *s*	—	—	2.13 *s*	2.18 *s*	2.00 6H, *s*	—	—	—
OH	not observed	—	—	not observed	2.86 *br s* (excl. D_2O)	—	—	5.20 1H, *br s*	—	—

dishielding effect and the small coupling constant value observed for H-6 in **1c** are in agreement with its location in an equatorial position where it makes approximately 60° angles with both C-7 protons. The ^{13}C NMR spectrum (Table 2) is also consistent with both the structural change and the model studies discussed above. Hence, only one non-hydrogenated carbon was found at δ43.6 and assigned to C-5. The γ-carbons, C-10 and C-8, should be shielded approximately 7 ppm relative to hydrocarbon **2**, by the axial hydroxyl group (see Table 2).

Silica gel column chromatography of the pooled fractions 23–35 yielded two oily compounds, homogeneous by TLC. The major one, **4**, showed a molecular ion at m/z 318 ($C_{20}H_{30}O_3$) and the minor one, **5**, had $[M]^+$ at m/z 402 ($C_{24}H_{34}O_5$).

Compound **4** showed a very similar ^{1}H NMR spectrum to that of **1**, except for an extra absorption around δ3.4. Their ^{13}C NMR spectra (Tables 2 and 3) were also very similar, except for an absorption at δ80.3 for **4**. As one can notice **4** is 16 mass units higher than **1** and must be a diol derivative of annonene. In order to confirm the diol nature of **4** chemically, we attempted its acetylation first in acetic anhydride–pyridine at room temp. and then under refluxing conditions. However, just a monoacetate (**4a**) which presented a molecular ion at m/z 360 ($C_{22}H_{32}O_4$) was obtained. Analysis of the ^{1}H NMR spectrum of **4a** revealed that an absorption, in comparison to **4**, had been deshielded, δ4.90 (1H, J = 10.4 and 11.2 Hz, H-7), while the other one remained at δ3.40 (1H, *d*, J = 10.4 Hz, H-6). A closed analysis of the splitting pattern and the J values of the absorption at δ4.90 shows that the methine hydrogen of the carbon whose hydroxyl was not acetylated (C-7) had two adjacent axial hydrogens. Moreover, the methine hydrogen of the carbon whose hydroxyl was not acetylated (C-6), appearing as a simple doublet, was indicative of at least one adjacent non-hydrogenated centre. These features can be accommodated into the annonene skeleton only if the hydroxyl groups are positioned in a vicinal fashion. Thus, positions C-6 and C-7 would be the sites for oxidation since, for the same reason as for **1**, the NMR data ruled out the oxidation of C-11 and C-12. Once the hydroxyl groups are positioned in a vicinal situation at C-6 and C-7, the observed J values (H-6/H-7 = 10.4 Hz and H-7/H-8 = 12.2 Hz) define their relative stereochemistry. Indeed, as can be seen in the structure **4**, the axial–axial positioning of H-6, H-7 and H-8 explains the coupling pattern and the easier acetylation of the equatorial hydroxyl at C-7. Once that hydroxyl is acetylated, it shields the equatorial hydroxyl at C-6, already sterically hindered by the angular methyl (C-19) and the vinyl methyl (C-18).

Treatment of **4a** with PCC yielded a ketone (**4b**) confirming the monohydroxy character of **4a**. The major changes for the ^{1}H NMR of **4b** compared to **4a** were the presence of only a simple doublet at δ5.36 (1H, J = 12.0 Hz) for H-7, instead of a pair of doublets, the disappearance of the doublet at 3.40, and the strong dishielding effect on the angular methyl δ1.53 (C-19), originally at δ1.13 in **4a**. Reduction of ketone **4b** with $NaBH_4$ followed by chromatographic separation gave a

Table 2. ^{13}C NMR spectral data comparison of compounds **1–3**, **1a**, **1b**, and **1c** (in $CDCl_3$)

C	3	2	1	Δδ1*	1b	Δδ2*	1c	Δδ3*	1a
1	18.4	18.3	18.0	−0.3	18.4	0.1	17.9	−0.4	17.9
2	27.1	27.0	26.7	−0.3	26.6	−0.4	26.9	−0.1	−26.5
3	137.7	120.5	122.0	1.5	123.6	3.1	125.3	4.8	122.7
4	142.2	144.4	143.8	−0.6	139.4	−5.0	140.6	−3.8	142.7
5	37.8	38.3	44.4	5.7	55.1	16.8	43.6	5.3	42.4
6	35.8	36.4	75.5	39.1	214.0	177.6	71.4	35.0	78.0
7	27.4	27.6	38.4	10.8	43.7	16.1	38.5	10.9	33.2
8	36.8	37.0	34.6	−2.4	41.2	4.2	28.8	−8.2	−34.2
9	39.5	38.7	38.6	−0.1	38.9	0.2	38.7	0.0	38.6
10	47.2	46.6	45.5	−1.1	50.4	3.8	39.1	−7.5	−45.8
11	46.0	36.4	37.9	1.5	39.2	2.8	33.7	−2.7	38.4
12	63.0	18.4	18.0	−0.4	18.3	−0.1	18.3	0.1	17.7
13	131.6	125.8	125.5	−0.3	125.0	−0.8	125.7	−0.1	125.5
14	108.5	111.0	110.9	−0.1	110.8	0.2	111.0	0.0	11.0
15	138.3	138.4	138.3	0.1	138.4	0.0	138.4	0.0	138.5
16	143.4	142.6	142.8	0.2	142.7	0.1	142.6	0.0	142.4
17	16.0	16.0	15.6	−0.4	16.1	0.1	15.5	−0.5	15.4
18	167.9	17.9	15.0	−2.9	18.7	0.9	17.4	−0.5	16.3
19	20.9	20.9	22.3	2.3	19.7	0.3	20.5	0.5	21.8
20	17.9	18.3	17.7	−0.6	19.1	0.8	18.0	−0.3	17.7

*$\Delta\delta m = \delta$Cn (**y**) − Cn (**2**), $m = 1, 2, 3$; **y** = **1**, **1b** and **1c**.

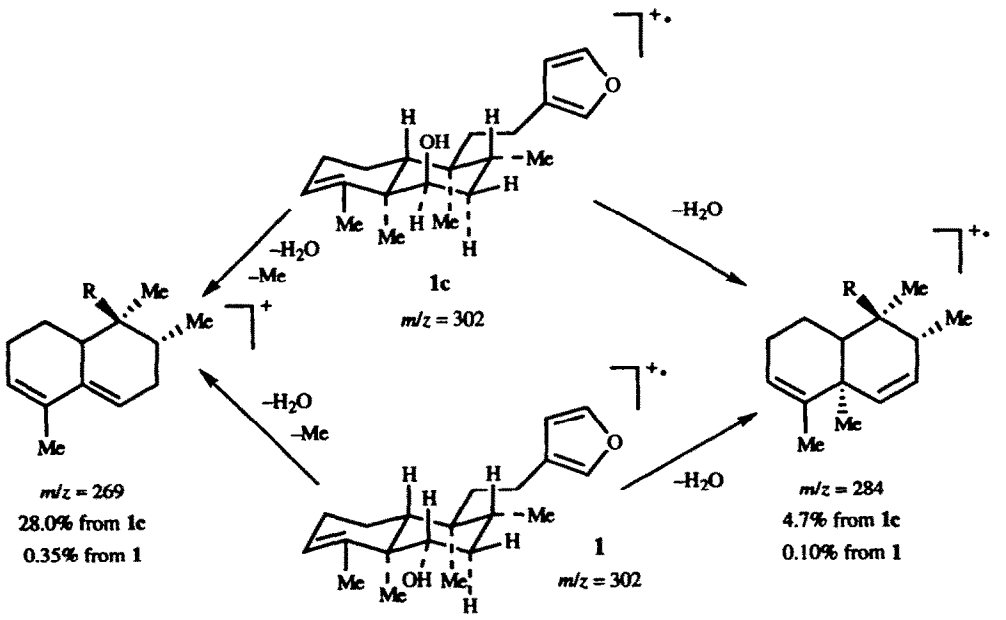
Table 3. NMR spectral data of compounds **4**, **4a**, **4b**, **4d**, **4f** and **5*** (in $CDCl_3$)

	^{13}C	^1H	^1H, ^1H-COSY†	^{13}C				
C	**4**	**4**		**4a**(acetone-d_6)	**4b**	**4d**	**4f**	**5**
1	17.8	1.60		18.4	18.2	20.0	21.8	18.4
2	26.4	2.05		27.0	26.4	27.0	27.0	27.2
3	122.0	5.23		122.3	123.7	128.6	126.0	123.8
4	143.4			144.8	138.9	132.2	134.1	142.2
5	43.4			44.7	54.6	58.2	57.5	43.9
6	80.3	3.20		78.0	207.2	205.2	201.4	79.1
7	74.7	3.50		78.0	76.5	204.9	120.2	75.2
8	40.9	1.50		40.8	44.8	44.8	142.3	40.5
9	39.0			39.9	39.4	43.8	39.5	39.9
10	44.8	1.40		45.7	49.8	53.0	44.0	45.8
11	38.7	1.50		39.8	39.0	36.0	38.1	39.8
12	17.7	2.20		18.4	18.2	21.0	19.3	18.1
13	125.2			126.0	124.7	125.0	120.9	126.0
14	110.8	6.19		111.7	110.8	110.8	112.6	111.7
15	142.6	7.35		143.3	142.9	143.0	140.7	143.6
16	138.3	7.19		139.3	138.5	138.6	148.8	139.5
17	11.0	1.03		11.2	12.0	9.7	23.3	11.1
18	16.4	1.86		17.0	18.2	15.2	15.0	17.9
19	22.3	1.08		22.0	20.6	22.6	24.7	21.5
20	18.9	0.80		19.2	18.5	19.1	19.3	19.2

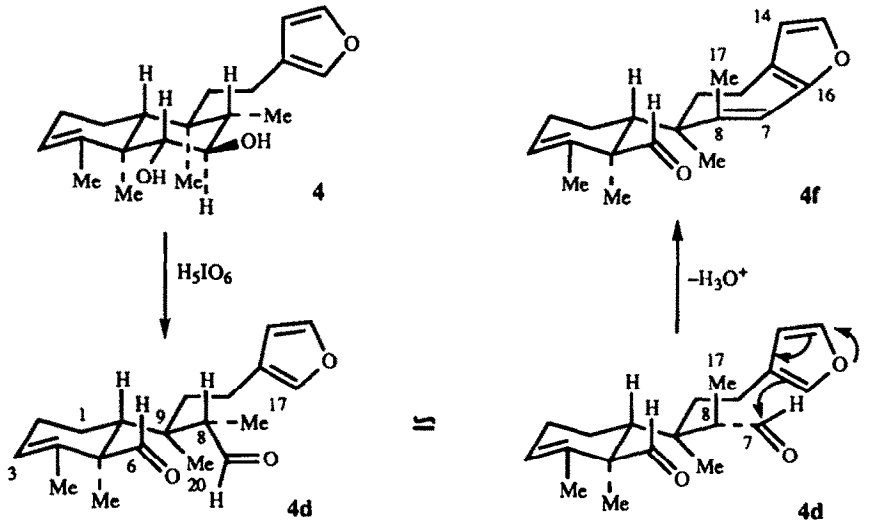
*The assignment for **4** was done based on DEPT, ^1H, ^1H-COSY and ^1H, ^{13}C-COSY experiments. For the other ones by comparison to **4** and chemical shift theory backgrounds.

†The ^1H, ^1H and ^1H, ^{13}C-COSY experiments were run on a Bruker AC-200 (200 MHz). Observed correlations are connected by arrows (↔).

monoacetate **4c**, characterized as the C-6 epimer of **4a** by mass spectrometry and ^1H NMR spectral comparison (Table 1). The new monoacetate showed similar mass and ^1H NMR spectra to those of **4a**, the principal differences being in the ^1H NMR spectrum showing a pair of doublets at δ5.09 (1H, $J = 3.0$ Hz and 11.0 Hz, H-7) and a doublet at 3.83 (1H, $J = 3.0$ Hz, H-6). These findings are in complete agreement with an axial hydroxyl at C-6, which



Scheme 1. Fragmentation pattern of 1 and 1c in the mass spectrometer.



Scheme 2. Suggested mechanism for the transformation of 4 into **4d** and **4f**.

leads to an equatorial–axial gauche interaction between H-6 and H-7, the later showing in addition, an axial–axial coupling to H-8.

The *trans*-equatorial–equatorial vicinal diol structural feature should undergo oxidative cleavage with periodate. When the diol **4** was oxidized with periodic acid in ether and the reaction mixture, after work-up, was poured into methanol, a precipitate was formed. After filtration the mother liquor was concentrated and chromatographed over a small silica gel column to provide a dialdehyde **4d**, characterized by ^{1}H and ^{13}C NMR analysis of its phenylhydrazone (**4e**) obtained readily upon treatment with 2,4-dinitrophenylhydrazine. Compound **4d** showed $[M]^+$ at *m/z* 316 ($C_{20}H_{28}O_3$) and the expected NMR data displayed in Tables 1 and 3.

The solid material that precipitated with methanol was recrystallized from methanol, to yield **4f**, mp 170° (with decomposition), $[M]^+$ at *m/z* 298 ($C_{20}H_{26}O_2$), IR 1710 cm^{-1} (C=O), 2700 and 2810 cm^{-1} (aldehyde Fermi resonances). Its ^{1}H NMR spectrum (Table 1) revealed the disappearance of an absorption for one hydrogen of the furan ring and the tertiary aldehyde absorption signal. The difference of 18 mass units for **4f**, compared to **4d**, was indicative of an intramolecular reaction wherein the furan

ring has undergone electrophilic attack by the aldehyde carbonyl at C-7 followed by dehydration, as depicted in Scheme 2. Thus, all spectroscopic and chemical data support the assignment of structure **4** as 6α,7β-dihydroxyannonene.

Compound **5**, was proven to be the diacetate of **4** in the following way. Even though we were unsuccessful in transforming **4** into **5** by acetylation, for the reasons explained above, we did get **4** from **5** by $LiAlH_4$ reduction. The obtained diol was identical to **4** in all respects after spectral and cochromatographic analysis.

Recently, Merritt and Ley [9] published a definitive paper about clerodane diterpenes. From their work it seems quite common for clerodanes to have either C-6 or C-7, or both, oxygenated. However, we have not found in the literature any report of **1**, **4** or **5**, or their derivatives. Thus, to the best of our knowledge, 6α-hydroxyannonene 6α,7α-dihydroxyannonene and 6α,7β-diacetoxyannonene are new natural secondary metabolites for the species and for the neo-clerodane diterpenes class.

EXPERIMENTAL

General. Mp uncorr. IR: as KBr pellets or neat films. UV: MeOH solns. Specific rotations ($[\alpha]_D$): as $CHCl_3$ solns. Circular dichroism: as MeOH solns. Mass spectra (EI, 70 eV) on a Finnigan 3200 GC-MS mass spectrometer coupled to an INCOS data system. ^1H NMR on either Varian EM-390 (90 MHz, CW) or Bruker AC-200 (200 MHz, FT). ^{13}C NMR on either Jeol JNM-FX60 (15.03 MHz, FT) or Bruker AC-200 (50 MHz, FT). Both ^1H and ^{13}C NMR were run in $CDCl_3$. For both, the chemical shifts are expressed in ppm relative to TMS as internal standard (δ).

Plant material. The entire roots of *Croton sonderianus* Muell. Arg. were used in this study. The whole plant was collected in Sobral, Ceará, Brazil, and identified by Dr Afrânio G. Fernandes (UFC). Voucher specimens representing the collection are deposited at Herbario Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction and isolation of constituents. For the initial partitioning of the crude extract see the previous papers [1, 2]. A portion of the neutral fraction, 20 g, was adsorbed on to 20 g of silica gel and chromatographed over 100 g of silica gel (230–400 mesh) initially by elution with pure hexane and finally with hexane–MeOH 55%. Similar fractions by TLC were combined and designated: F_0 (1.61 g), F_{1-2} (202 mg), F_3 (89 mg), F_{4-6} (62 mg), F_{7-12} (352 mg), F_{13-15} (640 mg), F_{16-17} (710 mg), F_{18-22} (2.84 g), F_{23-25} (2.98), F_{26-29} (2.27 g), F_{30-32} (2.90 g) and F_{33-35} (2.72 g). The less polar fractions yielded *trans*-annonene and *trans*-cascarillone [3].

6α-Hydroxyannonene (**1**). CC of fractions F_{16-17} and F_{18-22}, over silica gel for TLC, yielded 535 mg of **1**, homogeneous by TLC, as an oil: $[\alpha]_D$ −47.7° ($CHCl_3$; *c* 12.4); IR ν_{max}^{neat} cm^{-1}: 3420, 1500, 1450, 1390, 1030, 880. EI-MS *m/z* (rel. int.): 302 $[M]^+$ (1), 287 $[M-Me]^+$ (1), 269 $[M-Me-H_2O]^+$ (0.5), 257 $[M-Me-H_2CO]^+$ (1.5), ^1H and ^{13}C NMR see Tables 1 and 2.

6α-Acetoxyannonene (**1a**). Overnight treatment of **1** (200 mg) with 9 ml of Ac_2O–pyridine (2:1) and silica gel chromatography of the residue obtained after usual work-up, yielded 106 mg of **1a**, as an oil: IR ν_{max}^{neat} cm^{-1}: 11 720, 1500, 1450, 1260, 880. EI-MS *m/z* (rel. int.): 344 $[M]^+$ (1), 284 $[M-HOAc]^+$ (2), 269 $[M-HOAc-Me]^+$ (3), ^1H and ^{13}C NMR see Tables 1 and 2.

6-Oxoannonene (**1b**). Compound **1** (200 mg) was dissolved in CH_2Cl_2 and treated with a soln of 200 mg of PCC in 25 ml CH_2Cl_2, for 2 hr. Usual work-up and filtration of the reaction mixture over a small column of silica gel yielded, by elution with hexane, 150 mg of **1b**, as a yellowish oil: IR ν_{max}^{neat} cm $^{-1}$: 1705, 1500, 1450, 1165, 1030, 875. EI-MS *m/z* (rel. int.): 300 $[M]^+$ (1), 285 $[M-Me]^+$ (4), 272 $[M-CO]^+$ (2), 257 $[M-CO-Me]^+$ (7), ^1H and ^{13}C NMR see Tables 1 and 2.

6β-Hydroxyannonene (**1c**). Compound **1b** (120 mg) was dissolved in 10 ml of EtOH and stirred with excess of $NaBH_4$ in EtOH for 1 hr at room temp. The reaction mixture was diluted with 30 ml H_2O and extracted 3 × with 10 ml EtOAc. The EtOAc was dried over Na_2SO_4 and the solvent evapd to give a mixture of two alcohols where the 6β-epimer was the major product. Silica gel CC yielded 60 mg of **1c**, as an oil. IR ν_{max}^{neat} cm^{-1}: 3580sh, 3450, 1500, 1450, 1380, 875. EI-MS *m/z* (rel. int.): 302 $[M]^+$ (20), 284 $[M-H_2O]^+$ (5), 269 $[M-Me-H_2O]^+$ (28), 257 $[M-Me-H_2CO]^+$ (25), ^1H and ^{13}C NMR see Tables 1 and 2.

6α,7β-Diacetoxyannonnene (**5**). F_{23-25} was chromatographed over silica gel for TLC, in a similar way to the previous fraction, and yielded more **1** and 400 mg of a yellowish oil that was rechromatographed twice to yield 120 mg of a homogeneous oil by TLC, **5**. $[\alpha]_D$ −7.3° ($CHCl_3$; *c* 7.3); IR ν_{max}^{neat} cm^{-1}: 1740, 1500, 1400, 1375, 1250, 875. EI-MS *m/z* (rel. int.): 402 $[M]^+$ (10), 374 $[M-CO]^+$ (1), 342 $[M-HOAc]^+$ (2), 327 $[M-HOAc-Me]^+$ (1), 282 $[M-2HOAc]^+$ (21), 267 $[M-2HOAc-Me]^+$ (21), ^1H and ^{13}C NMR see Tables 1 and 3.

6α,7β-Dihydroxyannonene (**4**). F_{30-32} and F_{33-35} showed very similar ^1H NMR spectra and were combined. CC over silica gel by elution with $CHCl_3$–hexane (2:1) yielded **4**, 1.2 g, as a slightly yellow oil, $[\alpha]_D$ −22.2° ($CHCl_3$; *c* 5.5); IR ν_{max}^{neat} cm^{-1}: 3390, 1640, 1560, 1500, 1440, 1385, 1030, 875. EI-MS *m/z* (rel. int.): 318 $[M]^+$ (1), 303 $[M-Me]^+$ (0.5), 300 $[M-H_2O]^+$ (0.5), 285 $[M-Me-H_2CO]^+$ (1), 267 $[M-Me-2H_2O]^+$ (1), 257 $[285-CO]^+$ (0.5), 223 $[M-95(CH_2CH_2furyl)]^+$ (1), 205 $[223-H_2O]^+$ (7), 201 (6), 173 (12), 171 (4), 135 (23), 121 (28), 107 (38), 95 (72), 81 (100); ^1H and ^{13}C NMR see Table 3. Reduction of **5** (40 mg) with $LiAlH_4$ yielded a residue after work-up that was chromatographed over silica to yield 20 mg of a homogeneous oil showing the same TLC behaviour and spectral features as **4**.

6α-Hydroxy-7β-acetoxyannonene (**4a**). Compound **4** (420 mg) was submitted to usual acetylation with Ac_2O–pyridine (2:1) to yield 400 mg of **4a**, after usual work-up: $[\alpha]_D$ −7.0° ($CHCl_3$; *c* 1.6); IR ν_{max}^{neat} cm^{-1}: 360, $[M]^+$ (0.5), 345 $[M-Me]^+$ (0.5), 332 $[M-CO]^+$ (0.5), 330 $[M-HOAc]^+$ (6), 285 $[M-HOAc-Me]^+$ (11), 257 (4), 205 (22), 201 (41), 187 (44), 173 (245), 159 (38), 145 (33),

121 (66), 107 (59), 95 (85), 81 (100), ^{1}H and ^{13}C NMR see Tables 1 and 3.

6-Oxo-7β-acetoxyannonene (**4b**). Compound **4a** (380 mg) was stirred overnight with 350 mg PCC in 35 ml CH_2Cl_2. After usual work-up the reaction mixture was chromatographed over silica to yield 100 mg of the starting material (**4a**) and 140 mg of **4b**, as a yellowish oil. $[\alpha]_D$ −20.2° ($CHCl_3$; *c* 5.0); CD (MeOH; *c* 20.0): $\Delta\varepsilon_{293}$ −0.95, $\Delta\varepsilon_{221}$ +0.84; IR ν_{max}^{neat} cm^{-1}: 1743, 1500, 1380, 1250, 1065, 1030, 880. EI-MS *m/z* (rel. int.): 358 $[M]^+$ (3), 343 $[M-Me]^+$ (2), 298 $[M-HOAc]^+$ (2), 283 $[Me-HOAc-Me]^+$ (6), 265 $[283-Me]^+$ (4), 203 (20), 185 (74), 149 (52), 133 (66), 107 (73), 95 (100). ^{1}H and ^{13}C NMR see Tables 1 and 2.

6β-Hydroxy-7β-acetoxyannonene (**4c**). Compound **4b** (40 mg) was dissolved in 10 ml EtOH and stirred with excess $NaBH_4$ for 1 hr. Usual work-up yielded 35 mg of an oily residue that after chromatography afforded 20 mg of **4c**, as a yellowish oil, $[\alpha]_D$ +2.1° ($CHCl_3$; *c* 1.6); EI-MS *m/z* (rel. int.): 360 $[M]^+$ (6), 300 (3), 285 (5), 267 (10), 187 (63), 185 (56), 173 (47), 159 (55), 145 (72), 135 (61), 121 (90), 107 (100), 95 (79). ^{1}H and ^{13}C NMR see Tables 1 and 3.

6,7-seco-Annonene-6,7-dial (**4d**). Compound **4** (600 mg) was dissolved in 20 ml Et_2O, dried with Na, mixed with a soln of 600 mg of H_5IO_6 in Et_2O and then stirred for 1 hr while being monitored by TLC every 15 min. After this time, a 25 ml aliquot was taken washed with 20 ml H_2O followed by 20 ml of 50% aq. $NaHCO_3$ soln and finally with 20 ml satd NaCl soln. The Et_2O phase was dried over Na_2SO_4, the Et_2O evapd and the residue dissolved in MeOH. A ppt. formed which was removed by filtration. The filtrate was concentrated to yield more ppt. After filtration the filtrate was treated with a soln of 200 mg of 2,4-dinitrophenylhydrazine in 1.0 ml H_2SO_4 to obtain 250 mg of a solid orange mixture that was chromatographed over silica gel to yield 100 mg of an orange solid homogeneous by TLC, **4e**, mp 84–88°; EI-MS *m/z* (rel. int.): 496 $[M]^+$ (0.5), 463 $[M-H_2O-Me]^+$ (2), 401 $[M-95]^+$ (1), 383 $[401-H_2O]^+$ (1), 259 $[301-CH_2CO]^+$, 107 (35), 95 (56), 81 (100). The remainder of the periodate oxidation mixture was stirred for an additional 3 hr and then worked-up as described above. Comparison of the pptd material by TLC showed they were identical and thus combined. The MeOH from the mother liquor was evapd and the residue chromatographed over silica gel to yield 70 mg of a homogeneous dialdehyde (**4d**) as a yellowish, unstable oil characterized by NMR analysis. See Tables 1 and 3.

Compound **4f**. The combined ppts (40 mg) were redissolved in hot MeOH to yield 35 mg of a pale yellow amorphous solid (**4f**) mp 170–172° (with decomposition), $[\alpha]_D$ +185.0° ($CHCl_3$; *c* 1.6); CD (MeOH; *c* 3.0): $\Delta\varepsilon_{305}$ +3.91, $\Delta\varepsilon_{278}$ +7.53, $\Delta\varepsilon_{228}$ −4.52; UV λ_{max}^{MeOH} (log ε) nm: 278 (3.80), 295sh (3.62); IR ν_{max}^{KBr} cm^{-1}: 2810, 1710, 1500, 1450, 1070, 750; EI-MS *m/z* (rel. int.): 298 $[M]^+$ (8), 270 $[M-CO]^+$ (1), 161 $[M-137]^+$ (100), 143 $[161-H_2O]^+$ (21), 115 $[143-CH_2=CH_2]^+$ (37). ^{1}H and ^{13}C NMR see Tables 1 and 3.

Acknowledgements—The authors are grateful to Dr Francisco J. A. Matos (Pharmacognosist, Universidade Federal do Ceará) and Dr Afranio G. Gernandes (Botanist, Universidade Federal do Ceará) for plant collection and identification. This work was financially supported by grants from CAPES/CNPq/FINEP. E.R.S. acknowledges CNPq for the award of the Research Fellowship.

REFERENCES

1. McChesney, J. D., Clark, A. M. and Silveira, E. R. (1991) *J. Nat. Prod.* **54**, 1625.
2. McChesney, J. D., Clark, A. M. and Silveira, E. R. (1991) *Pharm. Res.* **8**, 1243.
3. McChesney, J. D. and Silveira, E. R. (1989) *Fitoterapia* **51**, 172.
4. McCrindle, R., Nakamura, E. and Anderson, A. B. (1976) *J. Chem. Soc., Perkin Trans 1* 1590.
5. McChesney, J. D. and Silveira, E. R. (1989) *Phytochemistry* **28**, 3411.
6. Roberts, J. D., Weigert, F. J., Kroschwitz, J. I. and Reich, H. J. (1970) *J. Am. Chem. Soc.* **92**, 1338.
7. Weigert, F. J. and Roberts, J. D. (1970) *J. Am. Chem. Soc.* **92**, 1347.
8. House, H. O. (1976) in *Modern Synthetic Reactions* 2nd Edn. W. A. Benjamin, Menlo Park.
9. Merritt, A. T. and Ley, S. V. (1992) *Nat. Prod. Rep.* **9**, 243.