

## STEROIDS AND TERPENOIDS FROM THE BARK AND THE WOOD OF *ENTANDROPHRAGMA CYLINDRICUM*

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(Received March 30, 1994)

**ABSTRACT.** Several steroids and terpenoids have been isolated from the wood and the stem bark of *Entandrophragma cylindricum*. The structure of the novel ergosta-5,24(28)-diene-7 $\alpha$ -OMe,3 $\beta$ -ol (1) has been assigned on the basis of spectroscopic data and chemical modification studies.

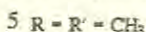
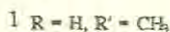
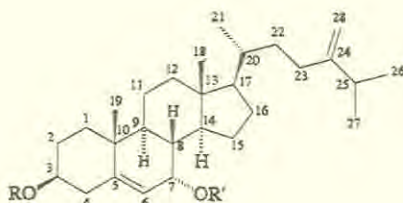
### INTRODUCTION

The genus *Entandrophragma* is widespread on the African continent, south of the Sahara, and occupies a prominent position in traditional African medicine. Five species are known in Cameroon and among these, *Entandrophragma cylindricum* Sprague (locally known as Sapele) has been the object of only limited chemical investigations. Previous work on the polar fractions of the methylene chloride extract of the stem bark and methanolic extract of the wood yielded three new acyclic triterpenoids named sapelenins A (2), B (3), C (4) [1], and a new flavonoid which was derived from pubeschin [2], respectively. New investigations focusing on the non polar fractions of the above mentioned extracts led to the isolation of a new ergosterol derivative, ergosta-5,24(28)-diene-7 $\alpha$ -OMe,3 $\beta$ -ol (1) and four steroids described in the literature [3-11], ergosta-5,24(28)-diene-3 $\beta$ ,7 $\alpha$ -diol (5), ergosterol peroxide (6), 3 $\beta$ -sitosteryl-heptadecanoate (7) and 3-O- $\beta$ -D-glucopyranosyl  $\beta$ -sitosterol (8) which are all derived from  $\beta$ -sitosterol (9), stigmasterol (10) and campesterol (11) isolated as a mixture. Two sesquiterpenes, (-)-globulol (12) and (-)-T-cadinol (13), and one triterpene, 22-hydroxy-hopane-3-one (14) have also been isolated during this study. The isolation and structural determination of these products are reported here.

### RESULTS AND DISCUSSION

Elemental analysis and mass spectral data agreed with the C<sub>29</sub>H<sub>48</sub>O<sub>2</sub> formula for compound 1. Observation in its mass spectrum of fragment ion at  $m/z$  410 [M-H<sub>2</sub>O]<sup>+</sup> suggests the presence of only one hydroxyl group. This is confirmed by the IR spectrum and by the preparation of a monoacetyl derivative (15). The <sup>13</sup>C NMR spectrum of 1 displayed signals for 29 carbon atoms: five methyls and one OMe ether, ten methylenes (9 sp<sup>3</sup>, 1 sp<sup>2</sup>), nine methines including one olefinic and two sp<sup>3</sup> carbons bearing oxygens, four quaternary carbons among these two sp<sup>2</sup> and two sp<sup>3</sup>. This is

confirmed by the  $^1\text{H}$  NMR spectrum in which six methyls were observed as three singlets and three doublets. The signal which appears at  $\delta 3.35$  is attributed to the OMe group due to the downfield shift. The proton geminal to this group appears at  $\delta 3.27$  and the one which is geminal to the hydroxyl group at  $\delta 3.60$ . The doublet and singlets observed at  $\delta 5.65$ ,  $4.65$  and  $4.75$  are due to an olefinic proton and to an exomethylene, respectively. The molecular formula  $\text{C}_{29}\text{H}_{48}\text{O}_2$  indicates six unsaturations and, based on the fact that compound **1** contains four  $\text{sp}^2$  carbon atoms (assigned to two double bonds) it is a tetracyclic compound. Comparison of the  $^{13}\text{C}$  and  $^1\text{H}$  NMR data of **1** (Table 1) with those of the literature [3-11] suggests that it is derived from ergosta-5,24(28)-diene-3 $\beta$ ,7 $\alpha$ -diol (**5**) by methylation of one of the two hydroxyl groups. This structural hypothesis is supported by the preparation of a monoacetate derivative (**15**) in which we observed the proton geminal to the acetate at  $\delta 4.70$  and an extra methyl due to the acetyl group.



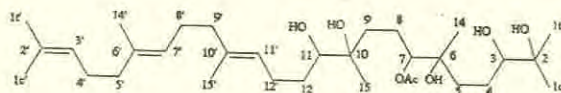
The COSY spectrum of compound **15** showed correlations between H-6 and H-4 on the one hand, and H-6 and the proton geminal to the methoxyl group on the other. The coupling between H-4 and the proton geminal to the acetate, and the absence of correlation between H-4 and the proton geminal to OMe indicate that the latter group is fixed in  $\alpha$  position of carbon C-7. Its  $\alpha$  orientation is proposed to account for the coupling constant between H-6 and H-7 [3-11]. The absence in the  $^{13}\text{C}$  NMR spectrum of a signal around  $\delta 81$  characterising the C-3 shift of 3 $\beta$ -OMe-steroids [12] brings further proof to the hypothesis.

HMBC correlations between the methyl of the methoxyl group and C-7, the other methyls and neighbouring carbon atoms allowed to establish connectivities among fragments of the molecule and confirm the structure **1**.  $\Delta^{4(E)}$  hypothesis was rejected after analysing the COSY spectrum of compound **15** including absence of a correlation between H-3 and the olefinic proton. Moreover, the presence of an  $\alpha$ -acetate in C-7 induced a characteristic  $^{13}\text{C}$  NMR signal around  $\delta 68$  [3-11].

Structures **2-4** were determined by means of spectroscopic data and chemical transformations [1] and those of compounds **5-8** and **12-14** by comparative analysis of physical and spectra data with those in the literature [3-11 and 13-17]. Compounds **9-11** were identified based on their GC and confirmed by addition of reference compounds. Their proportions are:  $\beta$ -sitosterol (**9**) 64%, stigmasterol (**10**) 21% and campesterol (**11**) 15%.

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for compounds **1** and **15**.

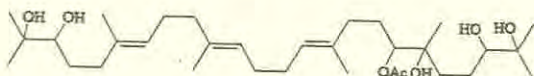
C	1	15	H	1	15
1	37.1	36.4			
2	31.4	27.5			
3	71.2	73.5	3	3.60 (m)	4.70 (m)
4	42.1	38.1			
5	146.2	145.0			
6	121.5	121.6	6	5.65 (d, $J=5.4$ Hz)	5.65 (d, $J=5.4$ Hz)
7	73.7	73.6	7	3.27 (m)	3.27 (m)
8	37.2	37.1			
9	42.4	42.5			
10	37.1	37.2			
11	20.6	20.7			
12	38.9	38.9			
13	42.0	42.0			
14	48.8	48.9			
15	24.1	24.1			
16	28.1	28.1			
17	55.5	55.6			
18	11.4	11.4	18	0.68 (s)	0.68 (s)
19	18.1	18.1	19	1.00 (s)	1.00 (s)
20	35.6	35.7	20	1.42 (m)	1.42 (m)
21	18.6	18.7	21	0.94 (d, $J=6.5$ Hz)	0.94 (d, $J=6.5$ Hz)
22	34.6	34.6			
23	30.9	30.8			
24	156.7	156.8			
25	33.7	33.8			
26	21.8	21.8	26	1.01 (d, $J=6.6$ Hz)	1.01 (d, $J=6.6$ Hz)
27	21.9	21.9	27	1.05 (d, $J=6.6$ Hz)	1.05 (d, $J=6.6$ Hz)
28	105.8	105.9	28	4.65 (s) & 4.75 (s)	4.65 (s) & 4.75 (s)
O-Me	56.5	56.6	O-Me	3.35 (s)	3.35 (s)
Ac		21.3 & 170.4	Ac		2.10 (s)



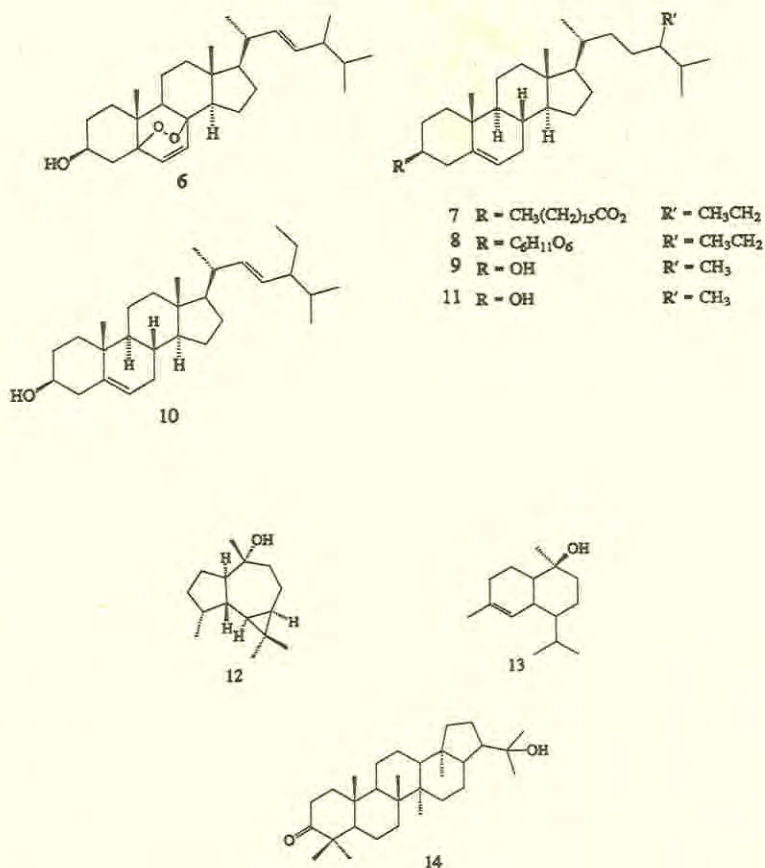
2



3



4



## EXPERIMENTAL

*General.* MPs uncorr. IR: NaCl. NMR spectra were recorded at 75.4 MHz for <sup>13</sup>C and 300.1 MHz for <sup>1</sup>H. Chemical shifts are given in δ values with TMS as an internal standard. <sup>13</sup>C chemical shifts are summarized in Table 1. EIMS were measured at 70 eV. GC: column and detector temperature are 80 and 330°, respectively. TLC was carried out on silica gel. Compounds were detected by spraying with 50% solution of H<sub>2</sub>SO<sub>4</sub> in H<sub>2</sub>O followed by heating.

*Plant material.* The bark and heartwood of *Entandrophragma cylindricum* were collected from Awae (Cameroon). A voucher specimen has been deposited at the National Herbarium, Yaounde.

*Extraction and isolation.* The air dried and finely powdered stem bark of *Entandrophragma cylindricum* (10 kg) was extracted with n-hexane (15 l) at RT. The residual powder was then extracted with methylene chloride (10 l) at RT. The

methylene chloride extract (33 g) was chromatographed on a silica gel column using methylene chloride-methanol gradient and collecting 150 ml fractions. Frs 20-44 and 86-100 were pooled (18 and 1.5 g, respectively) and rechromatographed repeatedly on a silica gel column using the same solvent system to yield a mixture of  $\beta$ -sitosterol (9), stigmasterol (10) and campesterol (11) and compound 6 in the frs 20-44, compounds 1 and 5 in frs 86-100.

The air dried, finely powdered wood of *Entandrophragma cylindricum* (5 kg) was extracted with MeOH (10 l) at RT. The MeOH extract (185 g) was stirred with  $\text{CH}_2\text{Cl}_2$  at RT to give solid and liquid phases. The liquid phase was filtered *in vacuo* over a sintered glass filter and concentrated using a rotatory evaporator to give the methylene chloride extract (15 g). This extract was chromatographed on a silica gel column using a  $\text{CH}_2\text{Cl}_2$ -MeOH gradient and collecting 100 ml fractions. Frs 1-50 and 91-181 were pooled (4 and 1 g, respectively) and rechromatographed repeatedly on silica gel column using the same solvent systems to yield compound 7 in frs 1-4 and compound 8 in frs 91-181.

**Compound 1.** (Found, C: 81.20, H: 11.19, O: 7.40  $\text{C}_{23}\text{H}_{48}\text{O}_2$ ; calculated, C: 81.30, H: 11.21, O: 7.49); mp 198°;  $[\alpha]_D -48$  ( $\text{Me}_2\text{CO}$ , c 0.1); IR  $\nu_{\text{max}}$  (NaCl)  $\text{cm}^{-1}$ : 3400 (OH), 1640 (double bond), 1250 (C-O);  $^1\text{H}$  and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ); see Table 1; EIMS  $m/z$  (rel. int.): 410 (1), 397 (3), 218 (20), 159 (35), 137 (100), 121 (90).

**Compound 15.** To a solution of 1 (5 mg) in  $\text{CH}_2\text{Cl}_2$  (5 ml) were added distilled  $\text{Ac}_2\text{O}$  (2 ml) dropwise with stirring and a catalytic amount of DMAP, and the mixture was stirred overnight at RT.  $\text{CuSO}_4$  solution (10%) was added and then the organic phase was washed with a saturated  $\text{NaHCO}_3$  solution and dried over  $\text{Na}_2\text{SO}_4$ . Co-evaporation with toluene was then performed and 15 was obtained as the sole compound.  $[\alpha]_D -45$  ( $\text{Me}_2\text{CO}$ , c 0.3); IR  $\nu_{\text{max}}$  (NaCl)  $\text{cm}^{-1}$ : 1740 (-OCOMe), 1640 (double bond), 1250 (C-O);  $^1\text{H}$  and  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ); see Table 1; EIMS  $m/z$  (rel. int.): 426 (1), 410 (50), 218 (15), 189 (40), 139 (100), 121 (90), 81 (80).

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