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# COMPLETE STRUCTURAL ASSIGNMENTS OF AN ERGOSTEROL DERIVATIVE FROM *ENTANDROPHRAGMA UTILE*

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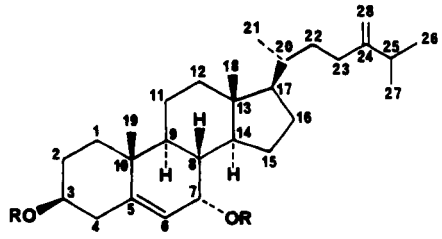
ABSTRACT.—On the basis of extensive structural analysis, a 3β,7α-dihydroxyergosta-5,24(28)-diene structure [**1**] was assigned to a compound isolated from *Entandrophragma utile*. This result necessitates the revision of a previously misassigned structure of a product isolated from the sponge *Haliclona oculata*.

*Entandrophragma utile* (Dawe et Sprague) Sprague (Meliaceae) is one of the five species of the genus *Entandrophragma* identified in Cameroon (1). The extracts from its bark revealed some preventive fungicidal effects against *Piricularia oryzae* (2) and are also used locally by traditional healers in the curing of malaria.

During the course of ongoing studies of the lipid fractions of the bark, we isolated a sterol to which, on the basis of physical data that will be detailed hereafter, we assigned structure **1**. A literature search then revealed that the very same structure had already been given to a marine sterol isolated from a finger sponge (3). However, as some physical data for the two compounds differ significantly from each other [for example, there is more than a 60° discrepancy between the melting points of the two compounds, and five resonances differ by some 3–6 ppm in their $^{13}$C-nmr spectra (3)], we now present evidence that **1** is indeed the correct structure for our plant-derived material.

## RESULTS AND DISCUSSION

Elemental analysis and high resolution mass spectra were in accord with the molecular formula $C_{28}H_{46}O_2$ for **1**. In the eims, two successive losses of 18 mass units from the low abundance molecular peak at *m/z* 414 suggested the presence of two hydroxyl groups. No other functionalities were present except for two double bonds, as evidenced by the lower field part of the $^{13}$C-nmr spectrum. A 300 MHz $^1$H-nmr spectrum, while confirming the above functionalities, additionally revealed five methyl groups: two were bound to quaternary carbons and one to a methine, and the last two belonged to an isopropyl system. DEPT-edited carbon spectra confirmed the presence of these five methyl groups and also disclosed ten methylene and nine methine carbons. Subtracting these various sub-spectra from the proton-decoupled carbon spectrum left



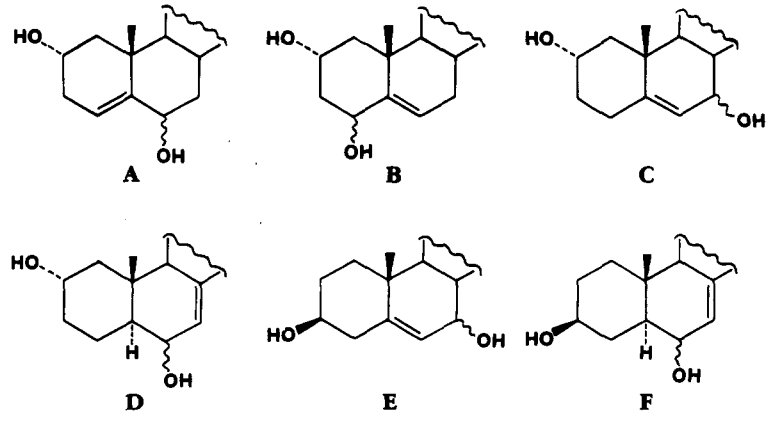

**1** R=H
**2** R=Ac

only three quaternary centers, an unexpected outcome in light of the gross formula. Hence, use was made of a quaternary specific pulse sequence (4) that revealed the fourth quaternary carbon, coincidentally obscured under a methylene resonance at 42.0 ppm.

Considering all of the above led us to point out a dihydroxylated bis-unsaturated sterol as a likely candidate structure.

Furthermore, on comparing the carbon side-chain assignments (5) of 24-methylene cholesterol with the resonances found for **1**, we were led to consider this skeleton for the basic structure. This was confirmed by the chemical shifts of the *exo*-methylene group and of the isopropyl moiety (6) in the $^1$H spectrum. Hence, we were left with two hydroxyl groups and one double bond to be arranged within the tetracyclic ring system.

Double resonance experiments allowed recognition of an allylic secondary alcohol (δ C*H*= 5.45 ppm, δ C*H*OH 3.7 ppm) which was not directly connected to the remaining secondary alcohol group. The complexity of the latter and its chemical shift ($\Delta \nu$ ½ = 22 Hz and δ C*H*OH 3.55 ppm) were as normally seen for a 3β-carbinol group although a 2α-substitution could also be possible (7). With regard to the location of the cyclic trisubstituted double bond, some positions could easily be excluded. A $\Delta^{16}$ double bond would induce shifts at the neighboring carbons of the side chain; this was not observed. Similarly, expected values (5) for a D ring were found for C-13, C-15, C-16, and C-17, which would otherwise undergo alpha or beta shifts. The presence of this double bond at positions 9(11) or 14 could also be excluded after considering the known chemical shifts for such alpha-hydroxylated unsaturated systems (8). Hence it was established that rings C and D were unsubstituted, thus leaving substructures **A**–**F** (both stereochemistries of the allylic group being considered) as the remaining candidates.




More could be learned after considering the carbon shifts observed upon acetylation of **1** to give diacetate **2**. In accord with precedent (9), downfield shifts were observed for carbons α to the acetyl group and upfield shifts for the β ones (Table 1). The multiplicity of the shifted beta carbons (two methylenes and two methines) led us to rule out substructures **A** and **B**. On the other hand, Zürcher type calculations (10), the results of which are displayed in Table 2, led us to eliminate $\mathbf{D}_\alpha$ and $\mathbf{F}_\alpha$ because deviations from the observed values (0.65 and 1.00 ppm, respectively, for the 18-Me and the 19-Me groups) are rather large.

Routine 2D nmr techniques were then used to gain further insight into the actual substitution pattern. Heteronuclear C/H correlation data were obtained and enabled the assignment of both alcohols. A similar experiment was then carried out using proton-relay coherence transfer (11,12). This disclosed in particular (Figure 1) that the

TABLE 1. $^{13}$C-nmr Data of Compounds **1** and **2**.

| Carbon | Multiplicity[a] | Compound | | Acetylation shifts[c] |
|---|---|---|---|---|
| | | **1** | **2**[b] | |
| C-1 | T | 37.0 | 36.6 | |
| C-2 | T | 31.2 | 27.6 | −3.6 |
| C-3 | D | 71.1 | 73.2 | +2.1 |
| C-4 | T | 42.0 | 37.9 | −4.1 |
| C-5 | S | 146.2 | 146.5 | |
| C-6 | D | 123.7 | 120.9 | −2.8 |
| C-7 | D | 65.3 | 68.3 | +3.0 |
| C-8 | D | 37.5 | 35.8 | −1.7 |
| C-9 | D | 42.2 | 43.1 | +0.9 |
| C-10 | S | 37.3 | 37.3 | |
| C-11 | T | 20.7 | 20.8 | |
| C-12 | T | 39.1 | 39.1 | |
| C-13 | S | 42.1 | 42.3 | |
| C-14 | D | 49.3 | 49.3 | |
| C-15 | T | 24.3 | 24.2 | |
| C-16 | T | 28.2 | 28.2 | |
| C-17 | D | 55.6 | 55.9 | |
| C-18 | Q | 11.6 | 11.5 | |
| C-19 | Q | 18.2 | 18.1 | |
| C-20 | D | 35.6 | 35.7 | |
| C-21 | Q | 18.7 | 18.7 | |
| C-22 | T | 34.6 | 34.7 | |
| C-23 | T | 30.8 | 31.2 | |
| C-24 | S | 156.8 | 156.8 | |
| C-25 | D | 33.7 | 33.7 | |
| C-26 | Q | 21.8[d] | 22.0[d] | |
| C-27 | Q | 21.9[d] | 22.1[d] | |
| C-28 | T | 105.9 | 106.0 | |

[a]Q = Me, T = $CH_2$, D = CH, S = C.
[b]Acetyl groups resonate at 21.2, 21.3, 170.4, and 170.7 ppm.
[c]Not quoted if less than 0.5 ppm.
[d]These assignments might be interchanged.

non-allylic alcohol was connected to a group located at 2.3 ppm. More information was obtained from the normal H/H COSY spectrum; besides previously detected couplings, the vinylic proton showed a correlation with other protons resonating at 2.3 ppm. These were in turn correlated with the non-allylic alcohol group, suggesting that

TABLE 2. Calculated Chemical Shifts for Me-18 and Me-19.

| Substructure | δ Me-18 | δ Me-19 |
|---|---|---|
| $\mathbf{C}_\alpha$ | 0.69 | 1.09 |
| $\mathbf{C}_\beta$ | 0.72 | 1.12 |
| $\mathbf{D}_\alpha$ | 0.54 | 0.85 |
| $\mathbf{D}_\beta$ | 0.58 | 1.06 |
| $\mathbf{E}_\alpha$ | 0.69 | 1.01 |
| $\mathbf{E}_\beta$ | 0.71 | 1.04 |
| $\mathbf{F}_\alpha$ | 0.54 | 0.80 |
| $\mathbf{F}_\beta$ | 0.58 | 1.01 |

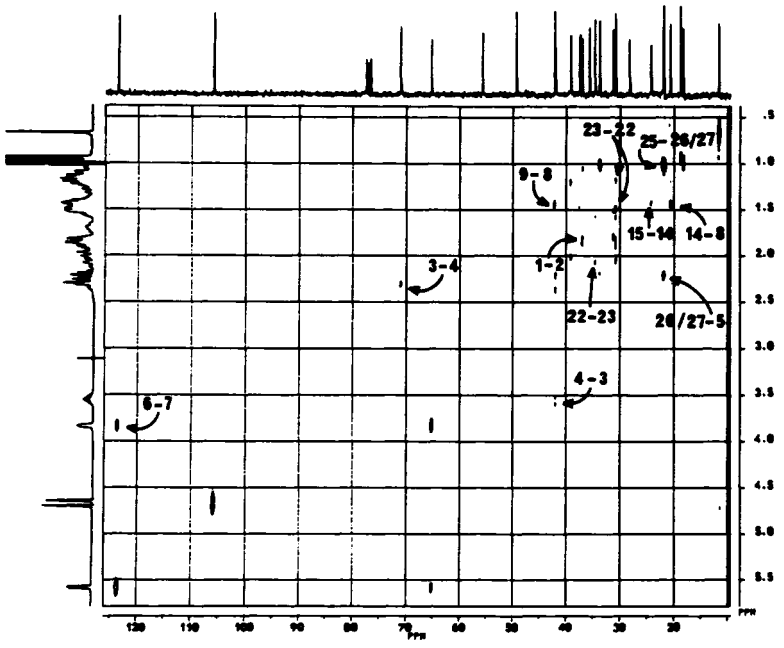



FIGURE 1. Heteronuclear proton relay transfer 2D experiment for **1**. Relay correlations have been assigned.

both alcohol groups were connected in some manner. However great care had to be exercised (13) with this interpretation because the 2.3 ppm region was crowded and different protons might have been involved in these correlations. A relay coherence H/H transfer (14,15) was set up; of particular interest was the new correlation obtained between the allylic alcohol and the 2.3 ppm region. This is indicative of a -CHOH-CH=C(R)-CH(R')-CHOH- array; but here again, for the reasons just stated, this needs confirmation. Hence some way had to be found to disentangle the 2.3 ppm region.

Diacetate **2**, though displaying the desired spreading (two protons at 2.35 ppm were clearly differentiated from a 1H multiplet at 2.2 ppm), now coincidentally showed the very same chemical shifts for both the *exo*-methylene group and the non-conjugated secondary acetate, both of which were coupled to the 2.2–2.35 ppm region. Double resonance experiments failed to clear the overall picture, so use was made of pyridine-induced shifts (16), a method that has been applied successfully in the steroid field. Indeed, quite large downfield shifts were observed for some protons (Table 3) when recording the spectrum of **1** in pyridine-$d_5$. Of particular interest was the 2-proton resonance at 2.7 ppm, due to geminal H-4 protons that showed (Figure 2) an H/H COSY correlation with both the non-conjugated alcohol and the vinylic proton. Only structure **E** can fit this requirement, which left only the correct stereochemistry at C-7 to be defined.

TABLE 3. Pyridine-Induced Shifts of **1**.

| Selected H resonances | δ ($CDCl_3$) | δ ($C_6D_5N$) | Δδ |
|---|---|---|---|
| H-6 . . . . . | 5.55 | 5.9 | −0.35 |
| H-7 . . . . . | 3.8 | 4.1 | −0.3 |
| H-3 . . . . . | 3.55 | 3.8 | −0.25 |
| H-4 . . . . . | 2.3 | 2.7 | −0.4 |

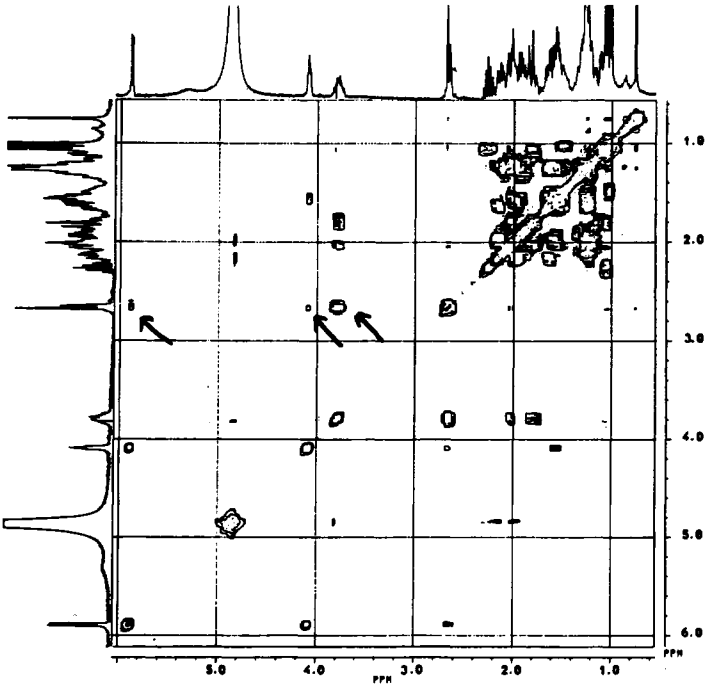
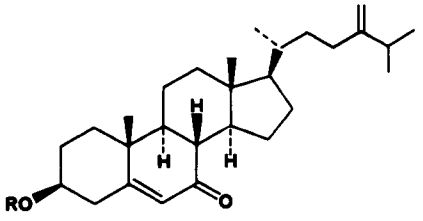



FIGURE 2. H/H COSY spectrum of **1** in pyridine; arrow shows correlations discussed in the text.

Gamma diaxial interactions with H-9 and H-14 are to be expected with a 7-OH in the α configuration [resulting in a large upfield shift for the corresponding carbons (8)]. This was easily observed in an uncrowded region of the carbon spectrum of **1** and left no doubt about the correctness of this stereochemistry. Further corroboration was observed by the "triplet" appearance (actually a double doublet with equal spacing, $J = 4.5$ Hz) of H-7 of diacetate **2**; a much larger coupling constant would have been expected for the 7-epimer.

A final confirmation of these results was obtained by chemical transformation of **1**. Selective allylic oxidation was accomplished by the use of pyridinium chlorochromate in the presence of pyrazole, an efficient procedure for oxidizing quasi axial alcohol groups (17). This gave **3** as the sole compound, which was best separated from pyrazole after acetylation. The acetate **4** thus obtained displayed spectroscopic data in complete agreement with the literature (3).

Full structural assignments for **1** are now straightforward. The $^{13}$C-nmr results can be found in Table 1, and, by means of the heteronuclear shift correlations, full proton assignments can then be deduced (Figure 3), resulting in complete structure elucidation.




**3** R=H
**4** R=Ac

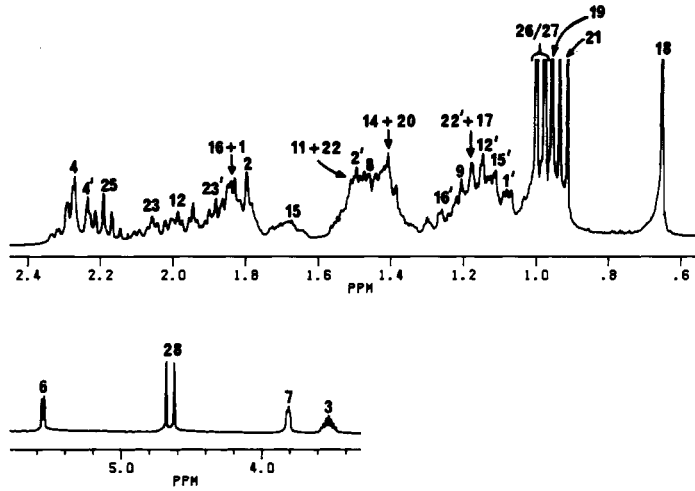



FIGURE 3. $^1$H-nmr (300 MHz) assignments of **1** in $CDCl_3$.

Structure **1** is now safely assigned to our plant-derived material. This necessitates the revision of the structure of the sponge-derived product (3).

Compound **1** is related to 7α-hydroxyfucosterol (18); other isomeric ergosterol derivatives can be found in the literature (19,20).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on Bruker AC 200 or AM 300 spectrometers operating at 200.13 MHz or 300.13 MHz for $^1$H and 50.32 MHz or 75.46 MHz for $^{13}$C. $^1$H-nmr chemical shifts were referenced to residual hydrogen absorptions of $CDCl_3$ (7.24 ppm) or of $C_6D_5N$ (8.71 ppm); coupling constants are given in Hz. $^{13}$C-nmr chemical shifts were referenced to $CDCl_3$ (77.0 ppm). 2D nmr correlation experiments (COSY 45, relay coherence transfer H/H COSY, XHCORR, and H/C RELAY) were performed using standard Bruker software. The mixing periods for relay experiments were 60 msec (ca. $1/1.6J_{H\text{-}Hmax}$) for H/H COSY (14) and 40 ms (ca. $1/4J_{H\text{-}H}$) for H/C RELAY (11). Low resolution mass spectra were obtained on a Nermag 10-10-C mass spectrometer at 70 eV. High resolution mass spectra were determined on a VG-Zab spectrometer. Melting point determination was performed on a Buchi-Tottoli apparatus using capillary tubes. Microanalyses were performed by the Service Central d'Analyse du CNRS, Solaize, France.

EXTRACTION AND ISOLATION.—*E. utile* was collected at Awae, near Akonolinga, South Cameroun, in January 1987. A voucher specimen has been deposited at the National Herbarium, Yaoundé. The air-dried and finely powdered stem bark of *E. utile* (10 kg) was extracted with *n*-hexane (15 liters) at room temperature. The defatted material was extracted with $CHCl_3$ (15 liters) at room temperature. The syrup thus obtained (60 g) was chromatographed on a Si gel 60 (70–230 mesh, 500 g) column using *n*-hexane/EtOAc mixtures of increasing polarity and collecting 200-ml fractions. Upon eluting with a 80:20 mixture, fractions 10–40 were obtained; these were rechromatographed using the same solvent system to yield pure **1** (300 mg, 0.5%).

*Ergosta-5,24(28)-diene-3β,7α-diol* [**1**].—*Anal.* calcd for $C_{28}H_{46}O_2$: C 81.12, H 11.11; found C 81.21, H 11.22. Eims *m/z* $[M]^+$ 414, $[M-H_2O]^+$ 396, $[M-2H_2O]^+$ 378; hrms *m/z* 378.3299 ($M-2H_2O$ requires 378.3287); mp 195°; $^{13}$C nmr see Table 1; $^1$H nmr see Figure 4.

*Ergosta-5,24(28)-diene-3β,7α-diol diacetate* [**2**].—To a solution of **1** (11 mg) in pyridine (1.5 ml) at −20° was added freshly distilled $Ac_2O$ (0.5 ml) dropwise with stirring, and the mixture was stirred at 4° for a few hours, then overnight at room temperature. MeOH was added to quench the excess of $Ac_2O$, and the solvent was evaporated under reduced pressure without heating. Coevaporation with 1,2-dichloroethane was then performed, and the crude reaction mixture was taken up in $CH_2Cl_2$ and washed with $H_2O$. After evaporation of the volatiles, **2** was obtained as the sole compound: $^{13}$C nmr see Table 1; $^1$H-nmr ($CDCl_3$, 300 MHz) 0.66 (s, $H_3$-18), 0.94 (d, $J=6.5$, $H_3$-21), 0.99 (s, $H_3$-19), 0.99 and 1.00 (two d, $J=6.2$, $H_3$-26 and $H_3$-27), 2.01 (s, OAc), 2.20 (h, $J=6.7$, H-25), 2.33 (d, $J=8.1$, H-4), 4.64 (s) and 4.66 (two s, H-27 and H-28), 4.94 (dd, $J_1=J_2=4.5$, H-7), 5.57 (d, $J=5.1$, H-6).

*3β-Acetyloxyergosta-5,24(28)-dien-7-one* [**4**].—Compound **1** (5 mg) was dissolved in a 2% solution of pyrazole in dry $CH_2Cl_2$ (1 ml) at 4° under argon. Pyridinium chlorochromate (6 mg) was added at once, and the mixture was stirred for 30 min, after which time tlc showed complete conversion of the starting material to a compound strongly active under uv light. A saturated aqueous NaCl solution was added, and the mixture was extracted with $CH_2Cl_2$. The resulting extracts were dried over anhydrous $Na_2SO_4$ and evaporated to dryness under reduced pressure without heating. Cc was performed on Si gel 60, eluting with MeOH-$CH_2Cl_2$ (2:98). The crude compound **3** was then taken up in $CH_2Cl_2$ and washed successively with 0.5 M aqueous HCl, aqueous $NaHCO_3$, and brine. After evaporation of the solvent the material was acetylated as described above and chromatographed on Si gel, eluting with MeOH-$CH_2Cl_2$ (2:98) to give pure **4**, whose spectral data were in agreement with literature values (3).

## ACKNOWLEDGMENTS

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NOTE ADDED IN PROOF: For a discussion of approaches to steroid structure elucidation, see: W.R. Croasmun and R.M.K. Carlson, *Methods Stereochem. Anal.*, **9**, 387 (1987).

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