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ENTILIN D, A HEPTANORTRITERPENOID FROM THE BARK OF *ENTANDROPHRAGMA UTILE*

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Key Word Index—*Entandrophragma utile*; Meliaceae; heptanortriterpenoid; entilin D.

Abstract—A new heptanortriterpenoid, entilin D, has been isolated from the bark of *Entandrophragma utile*. Its structure which is based on a rearranged, ring D cleaved heptanortriterpenoid skeleton, was assigned on the basis of extensive NMR experiments.

INTRODUCTION

In a search for antifeedants against storage pests, we examined a methanol extract obtained from bark of *Entandrophragma utile*. This tree belongs to the Meliaceae, a family which contains *Azadirachta indica* from which the very potent antifeedant azadirachtin was isolated. *E. utile* has already been shown to contain the protolimonoids sapelin A and a derivative of sapelin F (3α, 21,23R-trihydroxy-24S,25-epoxy, 20αH) [1, 2], as well as the four tetranortriterpenoids methyl angolensate (andirobin group) [3], utilin (phragmalin group) [3, 4], utilin B [5] and utilin C [6] and the three heptanortriterpenoids entilin A (**1**), entilin B (**2**) [7] and entilin C (**3**) [8]. Detailed examination of the methanol extract of the bark of *E. utile* by means of multiple analytical and preparative chromatographic procedures allowed us to isolate a new heptanortriterpenoid (**4**) which we have called entilin D.

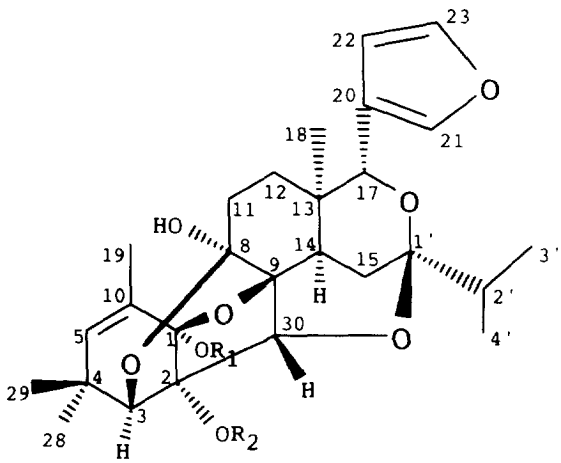
RESULTS AND DISCUSSION

The EI mass spectrum of **4** exhibited a molecular ion peak at *m/z* 530. This was confirmed by LSIMS. HR-mass spectrometry pointed to the formula $C_{29}H_{38}O_9$. The molecular ion undergoes two main primary fragmentations in which it loses molecules of water and acetic acid. The primary fragment ions undergo several further fragmentations yielding a very complex spectrum. The IR spectrum of **4** exhibited a hydroxyl band at 3493 cm^{-1} and a very strong carbonyl absorption at 1730 cm^{-1}, which can be assigned to the presence of an ester group or 6-membered cyclic ketone. Bands assignable to carbon–carbon double bonds were also visible. The UV spectrum of **4** exhibited two bands at λ_{max} 215 nm and 239 nm assignable to a furan and an α,β-unsaturated six-membered substituted ketone. All the above features were confirmed by the ^1H and ^{13}C NMR spectra of **4** which, with the complete assignments, are presented in Table 1.

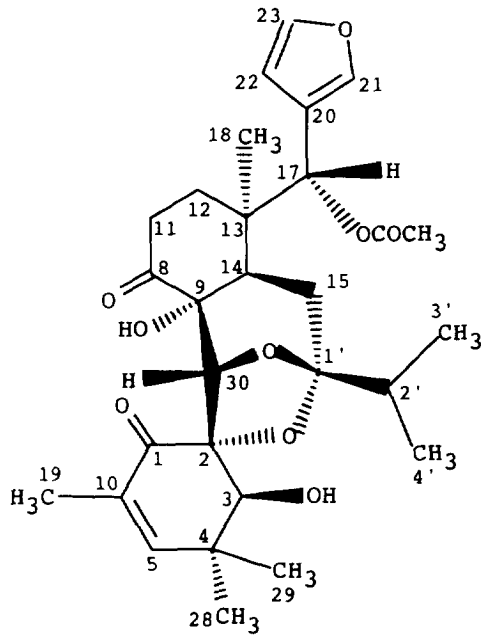
The NMR assignments were established by using several NMR techniques including the standard ^1H spectrum and single frequency decouplings for selected protons, fully decoupled and gated decoupled ^{13}C spectra, ^{13}C DEPT, ^1H–^1H COSY, ^1H–^{13}C COSY, long range ^1H–^{13}C correlation, and phase-sensitive NOESY spectra.

The molecular formula and some general spectral features of **4** suggested that this compound was an isomer of entilin B [7] with the carbon skeleton identical or very close to that of entilin B but with a different arrangement of the acetal and ester groups. The mass spectrum and NMR spectra of **4** indicated that it contained an acetoxyl group, a 3-substituted furan ring, one saturated and one α,β-unsaturated ketone moiety, two hydroxyl groups, an isopropyl group attached to a quaternary carbon atom and four methyl groups. There were also present two groups of strongly coupled hydrogen atoms, namely $-CH_2-CH_2-$ and $-CH-CH_2-$. It was assumed that the structure of **4** was similar to that of entilin B but with two opened hemiacetal rings; those involving the C-1 carbonyl group and 8-OH group, and the C-9 carbonyl and 3-OH groups. By using standard and long-range ^1H–^{13}C correlation spectra, it was possible to confirm the structure of the ring formed by carbon atoms 1–5 and 10 with attached methyl groups 19, 28 and 29. The chemical shift of the hydroxyl group on C-3 was easy to assign because of the significant coupling constant between the H-3 and 3-OH protons. The geometry of the substituents on C-2 and C-3 was assigned tentatively according to the known stereochemistry of entilin B.

The key step in the analysis of the structure of **4** was the presence of the long-range coupling between the



	R_1	R_2
Entilin A (**1**)	H	H
Entilin B (**2**)	H	Ac
Entilin C (**3**)	CH_3	H



Entilin D (**4**)

carbon atom of the carbonyl group of the acetoxyl substituent with the H-17 proton. The assignment of the latter was unequivocal due to the couplings with some of the protons and carbons of the furan ring. This observation indicated clearly that the acetoxyl group was attached to C-17, therefore requiring another arrangement of the acetal moiety built around C-1′. Careful examination of stereomodels showed that the only possibility was for the C-1′ and C-2 to be connected by an oxygen bridge. This finding led to full structure of **4** as shown in formula for **4**.

This structure was in full agreement with all of the available spectral data. The relative configuration of the chiral carbon atoms was confirmed by comparing the experimental and calculated ^1H–^1H coupling constants and by a NOESY experiment. It was established, for example, that the configuration of C-14 was as shown in **4** because only in this case were the experimental coupling constants $^3J_{H\text{-}14,H\text{-}15\alpha}$ and $^3J_{H\text{-}14,H\text{-}15\beta}$ in good agreement with the calculated ones. It was not possible to assign unequivocally the chemical shifts of H-11α and H-11β, and H-12α and H-12β because the conformation of the ring formed by C-8, C-9, C-11, C-12, C-13, C-14 is very flexible, as shown by molecular mechanics calculations. The stereochemistry of the acetal rings was confirmed by the NOESY experiment. Correlations between the 3-OH proton and CH_3-3′ and CH_3-4′, between CH_3-28 and H-30, and between H-3 and CH_3-29 can be explained only by the formula for **4**. The correlation between the 8-O<u>H</u> and CH_3-18 groups confirmed the relative stereochemistry of the C-8 and C-13 atoms. The only unknown is the relative configuration of C-17. However, it seems logical to propose the configuration presented in **4**, because this configuration is common to many tetranortriterpenoids and the entilins.

EXPERIMENTAL

The bark of *Entandrophragma utile* was collected in the primeval forest in Zaire near Kisangani in 1990. The tree was identified by Prof. F. Szafrański, University of Kisangani, according to [9]. The NMR data were obtained in $CDCl_3$.

E. utile dry bark (90 g) was exhaustively extracted with MeOH. The extract (10.8 g) was dissolved in a mixture of H_2O and Et_2O, and the H_2O layer extracted with Et_2O in a continuous extractor system. The combined Et_2O extracts (1.3 g) were chromatographed on silica gel in a 5 → 10% Me_2CO/C_6H_6 gradient solvent system. Fraction 1 eluted with 5% Me_2CO/C_6H_6 (600 mg) was rechromatographed in a 10% Me_2CO/C_6H_6 solvent system. Fractions 2 and 3 of the last chromatography were combined (232 mg) and rechromatographed using a 2 → 5% Me_2CO/C_6H_6 solvent system, fraction 3 (17 mg, R_f 0.35 10% Me_2CO/C_6H_6) contained pure **4** which was recrystallized from $CHCl_3$ to give crystals of entilin D (**4**), 1st mp 107–110°, 2nd 177–178°; $[\alpha]_D^{20}$ − 38.6° ($CHCl_3$; *c* 0.3); UV λ_{max}^{EtOH} nm: 216 (ε = 7140), 239 (ε = 5050); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3493, 1730 br. vs; ^{13}C and ^1H NMR: Table 1; HR-MS: $[M]^+$ 530.25213; calc. for $C_{29}H_{38}O_9$ $[M]^+$ 530.25157; MS 70 eV, *m/z* (rel. int.): 530 $[M]^+$ (9), 512 (3), 494 (1), 470 (11), 452 (8), 416 (7), 382 (5), 374 (12), 373 (12), 356 (11), 343 (17), 346 (7), 331 (9), 313 (6), 301 (20), 285 (15), 273 (26), 261 (30), 247 (16), 243 (22), 205 (100), 189 (17), 165 (28), 139 (28), 123 (24), 95 (20), 71 (40), 43 (48).

Table 1. ^1H and ^{13}C NMR data for compound **1**

C/H	δ_C	δ_H (J)
1	196.2 (*s*)	
2	88.4 (*s*)	
3	79.4 (*d*)	3.74 (*d*, J = 3.2 Hz)
4	38.7 (*s*)	
5	148.0 (*d*)	6.15 (*q*, J = 1.4 Hz)
8	214.9 (*s*)	
9	73.6 (*s*)	
10	133.4 (*s*)	
11	33.7 (*t*)	2.54 (*ddd*, J = 15.4, 9.0, 4.9 Hz)
		2.61 (*ddd*, J = 15.4, 10.6, 6.4 Hz)
12	29.2 (*t*)	1.35 (*dddd*, J = 13.8, 9.0, 6.4, 1.5 Hz)
		2.01 (*ddd*, J = 13.8, 10.6, 4.9 Hz)
13	38.9 (*s*)	
14	39.0 (*d*)	2.86 (*ddd*, J = 10.0, 8.8, 1.5 Hz)
15	29.3 (*t*)	1.82 (*dd*, J = 14.0, 10.0 Hz)
		2.15 (*dd*, J = 14.0, 8.8 Hz)
17	72.4 (*d*)	5.65 (*s*)
18	20.6 (*q*)	1.03 (*s*)
19	16.7 (*q*)	1.82 (*d*, J = 1.4 Hz)
20	121.5 (*s*)	
21	140.5 (*d*)	7.34 (*m*)
22	110.2 (*d*)	6.32 (*dd*, J = 1.9, 0.9 Hz)
23	142.7 (*d*)	7.36 (*t* J = 1.6 Hz)
28	22.5 (*q*)	1.24 (*s*)
29	30.0 (*q*)	1.22 (*s*)
30	80.7 (*d*)	4.65 (*s*)
1′	111.9 (*s*)	
2′	36.3 (*d*)	2.09 (*h*, J = 6.9 Hz)
3′	17.4 (*q*)	1.11 (*d*, J = 6.9 Hz)
4′	17.7 (*q*)	1.12 (*d*, J = 6.9 Hz)
–O<u>C</u>OCH$_3$	170.2 (*s*)	
–OCO<u>CH</u>$_3$	21.0 (*q*)	2.22 (*s*)
3-OH		2.45 (*d*, J = 3.2 Hz)
8-OH		3.32 (*s*)

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