## EXPERIMENTAL

The lichen was collected on tree trunks in El Valle Grande (alt 3000 m) near the town of Merida (MKF 314 on deposit in the herbarium of this faculty). TLC on Si gel $HF_{254}$ using $C_6H_6$–dioxane–HOAc (90:25:4) showed usnic ($R_f$ 0.6), norstictic ($R_f$ 0.42) and the fatty acid ($R_f$ 0.1). The fatty acid was detected by spraying with a soln of bromocresol green in EtOH (20 mg in 100 ml; 0.1 N NaOH added sufficient to give a blue colour). Subsequent spraying of the same plate with $H_2SO_4$ (10%) showed the aromatics with the same colours as previously reported [5]. Ground air-dried thallus (30 g) was successively extracted with petrol, $C_6H_6$, and $Me_2CO$ in a Soxhlet apparatus. The petrol and $Me_2CO$ extracts afforded usnic and norsticitic acids respectively. Concn of the $C_6H_6$ soln gave (**1**) as a white solid, mp 125° ($MeOH–H_2O$). $[\alpha]^{25}_{578}$ −15.4 (*c* 0.06, dioxane), $\nu^{KBr}_{max}$(cm$^{-1}$); 3500, 1730, 1705, 1680, 1250, 1215, 725, 715, PMR 60 Hz ($Me_2CO$, $\delta$): *ca* 1.4 (13 × $CH_2$), 2.1 (*s*, 3H), 2.43 (*t*, $CH_2$, $J = 7$ Hz), 2.63, 2.9, 3.07, 3.33 (AB *q*, 2H, $J = 16$ Hz), 2.65 (*t*, 1H), 3.65 (*s*, 3H), MS (*m/e*): 408 ($M^+$-$2H_2O$, 3%), 337 (15), 350 (11), 333 (7), 323 (11), 235 (5), 221 (9), 184 (12), 153 (4), 126 (6), 125 (15). (Found: C, 62.31; H, 9.11. $C_{23}H_{40}O_8$ requires: C, 62.12; H, 9%).

*DiMe ester of* (**1**). A soln of the acid (50 mg) in $Me_2CO$ (20 ml) was treated with excess $CH_2N_2$ to afford the tri Me ester (**2**) as a white solid, mp 41–44°. $\nu^{KBr}_{max}$(cm$^{-1}$): 3520, 1750–1715, 1230, 1160; $\delta$ ($CDCl_3$): *ca* 1.4 (13 × $CH_2$), *ca* 2.68 (*m*, 1H), 2.1 (*s*, 3H), 2.4 (*t*, $CH_2$, $J = 7$ Hz), 2.53, 2.8, 3, 3.33 (AB *q*, 2H, $J = 16$ Hz), 3.66, 3.71, 3.81 (*s*, 3H each). MS (*m/e*): 473 ($M^+ + 1$), 472 ($M^+$), 414, 413, 381 (413–32, *m** 351.1), 355, 349 (381–32, *m** 319.7), 323 (355–32. *m** 293.9), 101.

*Oxidation.* The acid **1** (100 mg) in $Me_2CO$ (20 ml) was treated with an $NaBiO_3$ (120 mg) and stirred at room temp. for 4 hr. The resulting clear soln was diluted with $H_2O$ and extracted with $Et_2O$. The soln was washed with $NaHCO_3$, $H_2O$, dried and evapd to yield a white solid (72 mg) which contained one major and two minor components. Chromatography on Si gel and elution with $CHCl_3$ gave the major component as white crystals, mp 85° (hexane). $\nu^{KBr}_{max}$(cm$^{-1}$): 1742, 1705, 1260, 1165; $\delta$ ($CDCl_3$): *ca* 1.4 (13 × $CH_2$), 2.13 (*s*, 3H), 2.45 (*t*, 2 × $CH_2$, $J = 7$ Hz), 3.33 (*s*, 2H), 2.65 (3H).

*Hydrolysis.* The acid **1** (50 mg) was dissolved in 5% KOH (10 ml) and stirred at room temp. for 4 hr. The soln was acidified and the ppt. recrystallized from $EtOH–H_2O$, mp 132–133°. $\nu^{KBr}_{max}$(cm$^{-1}$): 3480, 1760 *br*, 1270, 1254, 1238, 728, 715; $\delta$($C_5D_5N$): (13 × $CH_2$), 2.1 (*s*, 3H), 2.39 (*t*, 2H, $J = 7$ Hz), 3.33, 3.6, 3.8, 4.06 (AB *q*, 2H, $J = 16$ Hz), *ca* 3.5 (1H, partly obscured by quartet)

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# A NEW TRITERPENOID ALCOHOL FROM *TREMA ORIENTALIS*

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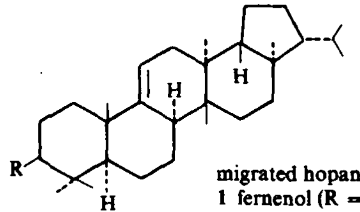
**Key Word Index**—*Trema orientalis*; Ulmaceae; new pentacyclic triterpenoid alcohol.

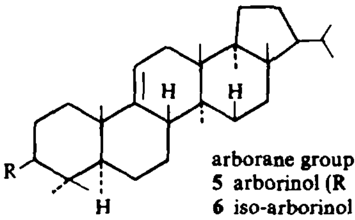
**Abstract**—Substances isolated from the stem-bark of *Trema orientalis* include a new pentacyclic triterpenoid alcohol, for which the name trematol is proposed. From its physical and chemical properties a partial structure is assigned, consisting of an arborane or a migrated hopane skeleton, with a 3$\beta$-hydroxyl group and minor modifications in rings D and E.

## RESULTS AND DISCUSSION

From the stem-bark of *Trema orientalis*, a reportedly [1] hypotensive plant, the triterpenes simiarenol [2], simiarenone [3], sitosterol and two other triterpenoid alcohols have been isolated. These two alcohols are very similar in polarity and were therefore quite difficult to isolate pure and free of contamination by each other. However, after very careful column chromatography over activated alumina, monitored by TLC and IR, followed by repeated recrystallizations, monitored by mp determinations and TLC, we were able to obtain the less polar alcohol, for which we propose the name trematol, as a pure crystalline solid, in very small quantity. The other, more polar, alcohol was also isolated in a very small



migrated hopane group
1 fernenol (R = —OH)
2 arundoin (R = —OMe)
3 fern-9(11)-ene (R = H)
4 simiarenol (R = —OH, 5(6) C = C)



arborane group
5 arborinol (R = – – – –OH)
6 iso-arborinol (R = —OH)
7 cylindrin (R = —OMe)
8 arbor-9(11)-ene (R = H)

quantity but not in a sufficiently pure form for reliable characterization and further studies.

Trematol is a colourless crystalline solid. Its IR spectrum (Nujol) showed bands a 3650 and 3496 (OH) and 820 cm$^{-1}$ (trisubstituted olefinic bond). Its NMR spectrum (60 MHz, $CDCl_3$, TMS) showed bands at the following $\delta$ values: 5.40 (broad, 1 vinyl proton), 3.45 (sharp singlet, 3α-hydrogen), 1.75–1.32 (broad complex multiplet, methylene and methyne protons), 0.99 and 0.95 (methyl protons), 0.89 and 0.85 (methyl protons), 0.80 (singlet, methyl protons), all integrating to 8 methyl groups and a total of 50 protons. The MS of trematol gave $M^+$ at $m/e$ 426 ($C_{30}H_{50}O$) with strong peaks at $m/e$ 411 ($M^+$-Me, base), 259 ($M^+$-167), 241 ($M^+$-167-$H_2O$) which are characteristic [4, 5] of $\Delta^{9(11)}$ and $\Delta^8$ unsaturated pentacyclic triterpenoids unsubstituted at $C_{12}$ [6]. However the IR and NMR spectra indicated the presence of an olefinic proton and hence rule out the possibility of a $\Delta^8$ type. The absence of a $\Delta^8$ function was further strengthened by a comparison of the changes in molar optical rotations [7] upon acetylation ($\Delta M_1$) and oxidation ($\Delta M_2$) of trematol with $\Delta^8$[8–11] and $\Delta^{9(11)}$ [5] compounds which contain a 3β-hydroxyl group (Table 1). All these data therefore suggest that trematol is a 9(11) unsaturated pentacyclic triterpenoid alcohol with a 3β-hydroxyl group.

Acetylation of trematol with acetic anhydride in pyridine gave a crystalline acetate with the expected NMR and IR spectra. The MS of the acetate gave $M^+$ at $m/e$ 468 and strong signals at $m/e$ 453 ($M^+$-Me, base), 393 ($M^+$-Me-HOAc), 301 ($M^+$-167) and 241 ($M^+$-167-HOAc). Trematol was readily oxidisable by Jones reagent to the ketone (IR) for which the name trematone is proposed. This reaction provides additional evidence for the presence of a secondary hydroxyl group in trematol. Trematone was obtained as a colourless crystalline solid, the MS of which gave $M^+$ at $m/e$ 424 ($C_{30}H_{48}O$) with strong peaks at $m/e$ 257 ($M^+$-167, base) and 409 ($M^+$-Me). It is noteworthy that, whereas in the MS of trematol the base peak occurred at $m/e$ ($M^+$-Me), in the spectrum of its ketone it occurred at $m/e$ ($M^+$-167). Trematone readily formed a crystalline 2,4-dinitro-phenylphydrazone.

Table 1. Optical rotation data for trematol and other triterpenes

| Structural type | $\Delta M_1^*$ | $\Delta M_2^*$ |
|---|---|---|
| $\Delta^8$ | +42 | +198 |
| $\Delta^{9(11)}$-ferneol | +33 | −93 |
| $\Delta^{9(11)}$-*iso*-arborinol type | +92 | −75 |
| trematol | +75 | −117 |

Judging from the structures of the triterpenes already isolated from the same extract, and in particular, from the fragmentation pattern of its MS, and the relative abundances of these signals compared with those of known structures [5], a fernane type structure with a 3β-hydroxyl group is proposed for trematol. Hence two structural types present themselves for further considerations. These are a migrated hopane type (1–4) or an arborane type (5–8) of pentacyclic triterpenes.

These two types of pentacyclic triterpenes have the same skeleton but are epimeric at C-8, C-13, C-14, C-17, C-18 and C-21. A comparison of the MS [5] of representative compounds from the two types of triterpenes with the MS of trematol and its functional derivatives (see Table 2) presents some interesting features:

(i) In all the compounds, the two strongest peaks occur at $m/e$ $M^+$-167 and $M^+$-15, the former being the base peak with the notable exceptions of arundoin (a methyl ether), trematol, and trematyl acetate, in each of which the base peak occurs at $m/e$ $M^+$-15.

(ii) The strong peaks at $m/e$ 241 in the MS of fernenol and trematol correspond to $M^+$-167-$H_2O$, while a similar peak at $m/e$ 241 in the spectrum of trematyl acetate corresponds to $M^+$-167-HOAc.

(iii) The MS of trematone is remarkably similar to that of arborinone with the base peak in both cases at $m/e$ $M^+$-167, except for a peak at $m/e$ 205 in trematone.

(iv) The MS of trematone and that of simiarenone [3], a $\Delta^5$ pentacyclic triterpene of the migrated hopane group, appear very similar from $m/e$ 245 downwards, but the MS of simiarenone interestingly lacks a strong peak at $M^+$-15.

(v) It is also interesting to note that in each of the MS of simiarenone and simiarenol [2] and glutinone [12] there is no strong peak corresponding to the loss of Me from the base peak ion (consisting of rings C, D and E) unlike in all the compounds listed above in which there is a pronounced loss of a Me from the molecular ion itself. Furthermore, in the MS of simiarenone [3] and simiarenol [2] there is a strong peak corresponding to the loss of an iso-propyl group from the base peak ion, whereas in the MS of all the compounds listed above there is no noticeable loss of an iso-propyl group from either the parent ion or any of the fragment ions. All these may have structural implications as to the detailed mechanism of fragmentation of these molecules on electron impact.

The remarkable similarity between the MS of trematone and arboronine led us to compare (Table 3) some other physical data of trematol and its functional derivatives with those of fernenol, arborinol and iso-arborinol [5]. From all available evidence, it is obvious that trematol cannot be any of these compounds nor the 3-

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*These figures represent the arithmetic mean [7] of the values of known members of the group with the particular stereo-skeleton.

Table 2. Mass spectral data (relative abundance %) of selected 9(II) pentacylic triterpenes

| *m/e* | 205 | 229 | 231 | 241 | 243 | 245 | 247 | 255 | 257 | 259 | 271 | 273 | 286 | 287 | 301 | 325 | 339 | 393 | 395 | 409 | 411 | 425 | 453 | $M^+$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ferneol (H)* | 10 | 17 | | 50 | | | 15 | 17 | | 100 | | 18 | | | | | | 25 | | | 87 | | | 30 | (426) |
| Trematol | | | | 30 | 15 | | 10 | | | 88 | | | 12 | | | | | 10 | | | 100 | | | 41 | (426) |
| Arundoin (H) | | | | | | | | | | | | 68 | | | | | | | | | | 100 | | 70 | (440) |
| Cylindrin (A) | | | | | | | | | | | | 45 | | 10 | | | | | | | | 76 | | 100 | (440) |
| Fern-9(II)-ene (A) | | | 17 | | 100 | | | | 20 | | | | | | | | | | 40 | | | | | 11 | (410) |
| Arbor-9(II)-ene (A) | | | 18 | | 100 | | | | 28 | | | | | | | 10 | | | 66 | | | | | 38 | (410) |
| Arborinone (A) | | | | | | 25 | | | 100 | | 38 | | | | | | 30 | | | 98 | | | | 62 | (424) |
| Trematone | 16 | | | | | 20 | | | 100 | | 25 | | | | | | 12 | | | 85 | | | | 52 | (424) |
| Trematyl acetate | | | | 50 | | | | | | | | | | | 90 | | | 40 | | | | | 10 | not meas. | (468) |

*H = migrated hopane group; A = arborane group.

Table 3. Physical constants of selected pentacyclic triterpenoid alcohols and derivatives [5, 13].

| Compound | alcohol | | acetate | | ketone | |
|---|---|---|---|---|---|---|
| | mp | $[\alpha]_D$ | mp | $[\alpha]_D$ | mp | $[\alpha]_D$ |
| Trematol | 215–216 | −27.8 | 244–246 | −9.1 | 219–220 | −55.4 |
| Arborinol | 274–275 | +34.2 | 242–243 | +12.7 | 214–216 | +28.8 |
| *Iso*-arborinol | 295–300 | +45 | 296–298 | +54 | 216 | +28.8 |
| Fernenol | 192–193 | −19.4 | 222–223 | −8.9 | 198–199 | −41.2 |

epimer of fernenol. Therefore, it appears that trematol is a new pentacyclic triterpenoid alcohol of either a migrated hopane or arborane skeleton but with slight structural modifications in rings D and E.

## EXPERIMENTAL

*Measurements.* Mps were taken using a liquid paraffin bath. Optical rotations were determined in $CHCl_3$. MS were run by Dr. E. S. Waight of Imperial College, London; while elemental analysis were carried out by both the staff of Imperial College, London, and University of Ibadan. TLC were run on Si gel G as adsorbent and visualized in $I_2$ vapour.

*Isolation of trematol.* Air-dried powdered stem-bark of the plant (10 kg) was extracted, in a Soxhlet apparatus, in 1 kg portions over 24 hr with petrol (bp 60–80°). The gummy extract (100 g) was triturated with $Et_2O$ and the $Et_2O$-insoluble fraction separated. The $Et_2O$ soln was fractionated into a neutral and acidic fraction with cold 10% aq. NaOH. The neutral fraction (70 g) was chromatographed over $Al_2O_3$ III and eluted in succession with petrol and petrol–$C_6H_6$ (1:1) and monitored by IR and TLC. The earlier fractions consisted mainly of derivatives of long-chain hydrocarbons. Further elution gave simiarenone followed by an unresolved mixture of alcohols which was eluted with $Et_2O$–MeOH (9:1). The crude mixture (30 g) of alcohols was rechromatographed over $Al_2O_3$ II. Careful elution with $C_6H_6$ gave simiarenol (350 mg) followed by a mixture of three other alcohols (TLC). The least polar of these, later identified as sitosterol, was well resolved on TLC from the other two, whose $R_f$ values were almost identical in several different solvent systems. The presence of these 2 alcohols was only clearly established by applying the technique of multiple TLC development in a relatively weakly polar solvent. The mixture of alcohols was rechromatographed on alumina II. Slow elution with $C_6H_6$ gave the two alcohols together unresolved finally followed by sitosterol. Separation of the alcohols on a preparative scale proved rather difficult. Attempts to separate them as their acetates or to separate the corresponding ketones were unsuccessful. The mixture was put on a long thin column of fully activated $Al_2O_3$ (Merck type H, 100 g $Al_2O_3$ per g of mixture) and slowly eluted with petrol–$C_6H_6$ (5:1, 2:1, and 1:1 successively) to give a crystalline fraction, predominantly trematol which, after repeated recrystallization from $CHCl_3$–MeOH, gave pure trematol as colourless crystals, mp 215–216°, [α] – 27.8 ($CHCl_3$) (Found: C, 84.19; H, 11.72. $C_{30}H_{50}O$ requires C, 84.44; H, 11.81%). Further slow elution with petrol–$C_6H_6$ (2:3) gave a crystalline solid which was predominantly the other alcohol (TLC) but which, after repeated recrystallizations from different solvents, had not been completely freed of trematol.

*Trematone.* A soln of trematol (41 mg) in $Me_2CO$ was treated with Jones reagent at room temp. and shaken until a dark-green ppt. appeared. The mixture was poured into excess $H_2O$ and extracted with $Et_2O$. The ethereal layer was washed several times with $H_2O$, dried ($Na_2SO_4$) and evapd *in vacuo* to give trematone (39 mg) as colourless plates mp 219–220° (from $CHCl_3$–MeOH), $[\alpha]_D$ – 55.4 (in $CHCl_3$).

*Trematone 2,4-dinitrophenylhydrazone.* A soln of trematone (10 mg) in $CHCl_3$ was treated with a MeOH soln of 2,4-dinitrophenylhydrazone hydrochloride and warmed to give a yellow crystalline ppt. which was recrystallized from $CHCl_3$–MeOH to give yellow crystals, mp 275°.

*Trematyl acetate.* Trematol (25 mg) in dry $C_5H_5N$ (0.5 ml) was treated with $Ac_2O$ (0.5 ml) at room temp. overnight. The soln was poured into excess $H_2O$ and extracted with $Et_2O$. mixture (30 g) of alcohols was rechromatographed over $Al_2O_3$ dried ($Na_2SO_4$) and evapd *in vacuo*. The residue was recrystallised from petrol (bp 60–80°) –MeOH to give needle-shaped crystals, mp 244–246°, $[\alpha]_D$ – 9.1°. (Found: C, 81.66; H, 11.20. $C_{32}H_{52}O_2$ requires; (C, 81.99; H, 11.18%).

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