Phytochemistry, Vol. 27, No. 7, pp. 2353–2355, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00
Pergamon Press plc.

A NEW LIMONOID FROM *TURRAEA ROBUSTA*

MOHAMED S. RAJAB, MICHAEL D. BENTLEY,* AHMED HASSANALI† and ANDREW CHAPYA†

Department of Chemistry, University of Maine, Orono, Maine U.S.A.; †ICIPE, Box 30772, Nairobi, Kenya

(*Revised received* 9 *November* 1987)

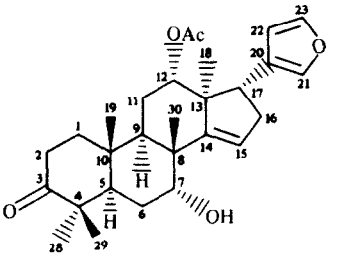
Key Word Index—*Turraea robusta*; Meliaceae; limonoid; tetranortriterpene; mzikonone; 12-α-acetoxy-1,2-dihydro-7-deacetylazadirone.

Abstract—A new tetranortriterpene, mzikonone (12α-acetoxy-1,2-dihydro-7-deacetylazadirone), has been isolated as the major limonoid from the root bark of *Turraea robusta* and its structure determined by spectroscopic and chemical methods. Its degree of oxidation is much lower than that of limonoids previously reported from two other *Turraea* species and this suggests that care must be taken in using the prieurianin and closely related limonoids as taxonomic markers for this genus.

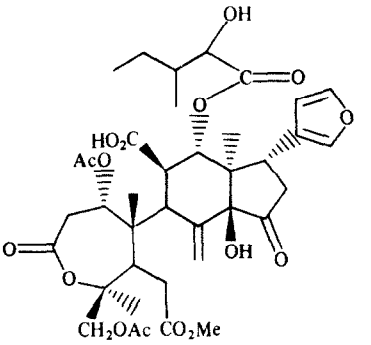
Turraea robusta Gürke (Meliaceae) is a tree found in East Africa. In the Zaramo (Tanzania) language, it is called mzikoziko, and in traditional medicine, a tea prepared from the roots is used to cure stomach pains and diarrhoea [1]. Extraction of air-dried root bark with methanol yielded an oil which was subjected to silica gel chromatography with a hexane–acetone gradient followed by a hexane–ethyl acetate gradient to yield mzikonone (**1**) as a white, microcrystalline solid (mp 99–101°). The structure of mzikonone was elucidated as 12α-acetoxy-1,2-dihydro-7-deacetylazadirone on the following evidence. High resolution mass spectrometry resulted in a parent ion of molecular formula, $C_{28}H_{38}O_5$. The IR spectrum (KBr) indicated the presence of an hydroxyl (3570 cm^{-1}), an ester (1740 cm^{-1}), a ketone (1715 cm^{-1}) and C–O (1250 cm^{-1}). The presence of the ketone and ester was further substantiated by ^{13}C NMR absorptions at δ 217 and 171 respectively. A three proton singlet at δ 1.91 in the ^1H NMR spectrum allowed assignment of the ester as an acetate. Application of ^{13}C NMR APT techniques resulted in assignment to mzikonone of six methyls, five methylenes, five saturated methines, four unsaturated methines, four quaternary saturated carbons, and two quarternary unsaturated carbons. Multiplet absorptions at δ 6.27, 7.23, and 7.35 in the 500 MHz ^1H NMR spectrum indicated the presence of a β-substituted furan. A triplet at δ 4.01 ($J = 2.8$ Hz) was consistent with assignment of the hydroxyl group to the 7-α position. The vinyl proton absorption at δ 5.68 (*dd*; $J = 2, 3$ Hz) was observed to shift upfield 0.2 ppm upon acetylation of the hydroxyl, consistent with assignment of the hydroxyl to the 7-α and the double bond to the C-14 position. A triplet at δ 5.10 ($J = 9$ Hz) led to assignment of the acetate of **1** to the C-12 position. Treatment of **1** with benzene seleninic anhydride resulted in an α,β-unsaturated ketone having ^1H NMR absorptions at δ 7.05 (*d*, $J = 12$) and 5.90 (*d*, $J = 12$), consistent with assignment of the ketone to the C-3 position. ^1H NMR COSY techniques verified coupling of H-17 and H-15 with H-16, H-9 and H-12 with H-11, H-22 with H-21 and H-23, H-1 and H-2, and H-6 with H-5 and H-7. Stereochemical assignments were verified with NOESY techniques. Particularly relevant were correlations between H-21, H-22, H-23 and the acetate methyl. These, in addition to an H-12–H-17 correlation proved the furan and the acetate were either both α or both β. The 30-Me was identified by correlation with H-7 and H-15. Correlation of H-12 with the 30-Me, confirmed α-stereochemistry for both the furan and the acetate. ^1H NMR and ^{13}C NMR chemical shifts, assigned with the aid of homonuclear (^1H–^1H) and heteronuclear (^1H–^{13}C) correlation NMR spectroscopy, are presented in the Experimental section.

Limonoids from two other species of *Turraea* have been reported [2]. Prieurianin (**2**) was isolated from *T. floribunda* and **3**, **4**, and **5** were isolated from *T.* obtusifolia. The latter limonoids are related to havanensin and heudelottin, which are limonoids of the genus *Trichilia* [3], and are intermediates enroute to the prieurianin group. It is of interest that mzikonone, as the principal limonoid of *T. robusta*, is much less oxidized than the limonoids **2**–**5**. This suggests that caution needs to be exercised in defining the oxidation pattern of limonoids which may be used as taxonomic markers for the genus *Turraea*. We are currently examining minor polar components in *T. robusta* extracts in a search for more highly oxidized limonoids. It is also worth noting that C-ring oxidation at C-12 alone in the meliacin class of limonoids is uncommon and we are aware of only one other example recently reported as a component of *Trichilia roka* [4].

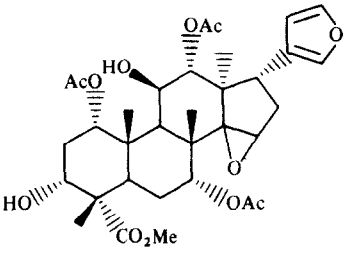
*Author to whom correspondence should be addressed.



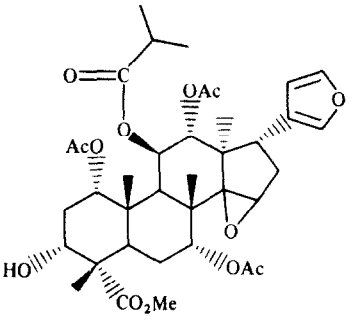
1



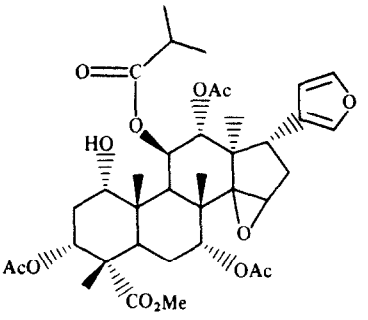
2



3



4



5

EXPERIMENTAL

Plant material. The root bark of *T. robusta* was collected in Awasi, Kismu District, Kenya, in September 1985. The plant was identified by Mr S. G. Mathenge (Department of Botany herbarium, University of Nairobi) and a voucher specimen is deposited in that department.

Extraction and isolation. T. robusta root bark (2.0 kg) was air-dried, powdered, and allowed to stand in MeOH (4 l) at room temp. for 1 week. The extract was filtered and evapd to dryness under vacuum to yield an oil (40 g). A portion of the oil (5.0 g) was chromatographed on silica gel (50 × 3.5 cm; 70–130 mesh) using a hexane–Me_2CO (1:4) to Me_2CO gradient. The oily fraction containing crude mzikonone (1.2 g) was rechromatographed on another silica gel column (50 × 2 cm; 230–400 mesh) eluted with an hexane–EtOAc gradient (15–30% EtOAc) to yield pure **1** (0.72 g) as a colourless solid, mp 99–101°. (Found: *m/z* 454.2705; $C_{28}H_{38}O_5$ requires *m/z* 454.2720). IR ν_{max}^{HBr} cm^{-1}: 3570 *br* (OH), 1740, 1715 (C=O) and 1250 (C–O). UV λ_{max}^{EtOH} nm: 215; ^1H NMR (500 MHz, $CDCl_3$): δ 1.034 (3H, *s*, 29-Me), 1.049 (3H, *s*, 18-or 19-Me), 1.054 (3H, *s*, 18-or 19-Me), 1.105 (3H, *s*, 28-Me), 1.160 (3H, *s*, 30-Me), 1.45 (1H, *m*, H-11α) 1.50 (1H, *m*, H-1β) 1.78–1.88 (3H, *m*, H-1α, H-6), 1.91 (3H, *s*, Ac), 2.07 (1H, *dd*, J = 12, 3 Hz, H-5), 2.2 (2H, *m*, H-9, H-11β), 2.4 (2H, *m*, H-2), 2.55 (2H, *m*, H-16), 3.05 (1H, *dd*, J = 8, 11 Hz, H-17), 4.01 (1H, *t*, J = 2.8 Hz, H-7), 5.10 (1H, *t*, J = 9, H = 12), 5.68 (1H, *dd*, J = 2, 3 Hz, H-15), 6.27 (1H, *m*, H-22), 7.23 (1H, *m*, H-23) and 7.35 (1H, *m*, H-21); ^{13}C NMR (125 MHz, $CDCl_3$): δ 216.9 (C-3), 170.8 (Ac), 158.7 (C-14), 142.1 (C-23), 140.2 (C-21), 124.3 (C-20), 122.5 (C-15), 111.6 (C-22), 77.2 (C-12), 71.8 (C-7), 51.3 (C-13), 50.2 (C-17), 46.9 (C-8), 46.3 (C-5), 43.9 (C-4), 42.6 (C-9), 38.4 (C-1), 37.0 (C-10), 36.8 (C-16), 33.8 (C-2), 25.3 (C-11), 24.8 (C-6), 21.3 (ACMe),

27.4 (30-Me), 26.1 (28-Me), 21.1, 15.6 and 14.8 (18-, 19- and 29-Me).

Acetylation of compound **1**. Acetylation of **1** (Ac_2O–pyridine, 50°, 24 hr) gave 7α,12α-diacetoxy-1,2-dihydroazadirone. EIMS *m/z*: 496[M]$^+$, 436[M−AcOH]$^+$, 376[M−2AcOH]$^+$. IR ν_{max}^{KBr} cm^{-1}: 1740, 1720 (C=O) and 1250 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.02 (6H), 1.04 (3H), 1.08 (3H), 1.19 (3H) [5 × Me], 1.91 (3H, *s*, 12Ac), 2.00 (3H, *s*, 7α-Ac), 2.99 (1H, *dd*, *J* = 9, 11 Hz, H-17), 5.07 (1H, *dd*, *J* = 7.8 Hz, H-12) 5.27 (1H, *m*, H-15), 5.48 (1H, *t*, *J* = 2.5 Hz, H-7), 6.27 (1H, *m*, H-22), 7.21 (1H, *m*, H-23) and 7.34 (1H, *m*, H-21).

Reaction of compound **1** with benzene seleninic anhydride. Compound **1** (100 mg) and benzene seleninic anhydride (80 mg) were refluxed for 30 min in chlorobenzene and the product, 12α-acetoxy-7-deacetylazadirone, purified by prep. TLC (silica gel, 20% EtOAc–hexane). EIMS *m/z*: 452[M]$^+$, 392[M−AcOH]$^+$, IR ν_{max}^{KBr} cm^{-1}: 3540 *br* (OH), 1730, 1670 (C=O), 1100 (C–O). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.05 (3H), 1.09 (3H), 1.17 (3H), 1.19 (3H) [5 × Me], 1.93 (3H, *s*, Ac), 3.08 (1H, *dd*, *J* = 9, 10.8 Hz, H-17), 4.04 (1H, *t*, *J* = 2.8 Hz, H-7), 5.16 (1H, *dd*, *J* = 8.6, 9 Hz, H-12), 5.70 (1H, *dd*, *J* = 2, 3 Hz, H-15), 5.82 (1H, *d*, *J* = 10 Hz, H-1) 6.28 (1H, *m*, H-22), 7.02 (1H, *d*, *J* = 10 Hz, H-2), 7.23 (1H, *m*, H-23) and 7.36 (1H, *m*, H-21).

Acknowledgements—The authors thank Dr Ben Shoulders of the University of Texas for obtaining NMR spectra and the USDA (Grant 85-FSTY-0102) for financial support.

REFERENCES

1. Kokwaro, J. O. (1976) *Medicinal Plants of East Africa*, p.158. East African Literature Bureau, Nairobi, Kenya.
2. Akinniyi, J. A., Connolly, J. D., Mulholland, D. A., Rycroft, D. S. and Taylor, D. A. H. (1986) *Phytochemistry* **21**, 2187.
3. Taylor, D. A. H. (1983) in *Chemistry and Chemical Taxonomy of the Rutales* (Waterman, P. G. and Grundon, M. F., eds), p. 138. Academic Press, London.
4. Nakatani, M., Iwashita, T., Mizukawa, K. and Hase, T. (1987) *Heterocycles* **26**, 43.

Phytochemistry, Vol. 27, No. 7, pp. 2355–2357, 1988.
Printed in Great Britain.

0031–9422/88 $3.00 + 0.00

PREPHYTOENE ALCOHOL FROM *MYRIOPHYLLUM VERTICILLATUM**

PIETRO MONACO, MARINA DELLA GRECA, MARGHERITA ONORATO and LUCIO PREVITERA

Department of Organic and Biological Chemistry of the University Via Mezzocannone 16, 80134 Napoli, Italy

(*Received* 20 *October* 1987)

Key Word Index—*M. verticillatum*; Haloragaceae; carotenoids; prephytoene alcohol.

Abstract—Prephytoene alcohol, a biosynthetic intermediate of carotenoids, has been isolated from the aquatic plant *Myriophyllum verticillatum*.

In a previous paper [1] we reported the isolation from the aquatic plant *M. verticillatum* of two novel hydroxylated carotenoids which were attributed structures **1** and **2** on the basis of their chemical and physical features. These compounds may easily be considdred to arise through water addition to the cyclopropylcarbinyl cation intermediate produced by loss of pyrophosphate from prephytoene pyrophosphate (**3b**).

In pursuing the chemical investigation of this species we isolated a compound in small amount from the ethereal extract which has been identified as prephytoene alcohol (**3a**) on the basis of its spectroscopic features.

Compound **3a** had $[\alpha]_D$ + 37.5° and a molecular formula $C_{40}H_{66}O$. The IR spectrum indicated the presence of a hydroxyl group and isolated double bonds with absorptions at 3400 and 1660 cm^{-1}. The mass spectrum showed, beside the molecular peak at *m/z* 562, fragments at *m/z* 544 [M−H_2O]$^+$, 531 [M−CH_2OH]$^+$, 529 [M−H_2O−Me]$^+$, and the series of fragments due to allylic cleavages in the side chains at *m/z* 493 [M−C_5H_9]$^+$, 475 [M−H_2O−C_5H_9]$^+$, 425 [M−$C_{10}H_{17}$]$^+$, 407 [M−H_2O−$C_{10}H_{17}$]$^+$, 357 [M−$C_{15}H_{25}$]$^+$, 339 [M−H_2O−$C_{15}H_{25}$]$^+$, 271 [M−H_2O−$C_{20}H_{33}$]$^+$, 203 [M−H_2O−$C_{25}H_{41}$]$^+$, 137 [$C_{10}H_{17}$]$^+$ and 69 [C_5H_9]$^+$. The H NMR spectrum showed the presence of methyl singlets at δ1.15, 1.61 and 1.69 in a 1:6:3 ratio, a

*Part 8 in a series of studies on aquatic plants distributed in Italy. For part 7 see ref. [1].