Phytochemistry, Vol. 20, No. 12, pp. 2753–2755, 1981.
Printed in Great Britain.

0031–9422/81/122753–03 $02.00/0

1′,2′-DIDEACETYLBORONOLIDE, AN α-PYRONE FROM *IBOZA RIPARIA*

LUC VAN PUYVELDE,*§ NORBERT DE KIMPE,† SERGE DUBÉ,* MONIQUE CHAGNON-DUBÉ,* YVES BOILY,* FRANÇOIS BORREMANS,‡ NICEAS SCHAMP† and MARC J. O. ANTEUNIS‡

*Centre Universitaire de Recherche sur la Pharmacopée et la Médicine Traditionelle, Curphametra, BP 52, Butare, Rwanda; †Laboratory of Organic Chemistry, Faculty of Agricultural Sciences, State University of Gent, Coupure 533, B-9000 Gent, Belgium; ‡Laboratory of Organic Chemistry, NMR Spectroscopic Unit, Faculty of Sciences, State University of Gent, Krijgslaan 271 (S4), B-9000 Gent, Belgium

(*Revised received* 30 *April* 1981)

Key Word Index—*Iboza riparia*; Labiatae; new α-pyrone; 1′,2′-dideacetylboronolide; sterols; sitosterol; stigmasterol; campesterol; structural determination.

Abstract—The structural elucidation of 1′,2′-dideacetylboronolide, 5,6-dihydro-6-(3′-acetoxy-1′,2′-dihydroxyheptyl)-2-pyrone, a new α-pyrone isolated from the leaves of *Iboza riparia* has been performed. Additionally, three sterols, sitosterol, stigmasterol and campesterol, have been identified in this species.

INTRODUCTION

In the course of investigations directed towards the isolation and structural determination of biologically active substances from medicinal plants of Rwanda, we have found significant pharmacological activity in extracts of the leaves of *Iboza riparia*, known locally as Umuravumba. The medicinal value of this species is well known among the native people of Rwanda [1] and, accordingly, several medicinal applications have been reported in Rwanda [1, 2] and elsewhere in eastern Africa [3].

In recent years, some attention has been devoted to constituents of *Iboza riparia*. Several compounds, among others ibozol [4], 7α-hydroxyroyleanone [4], umuravumbolide (**2**) [5], deacetylumuravumbolide (**4**) [5] and deacetylboronolide (**3**) [5], have been isolated from this species.

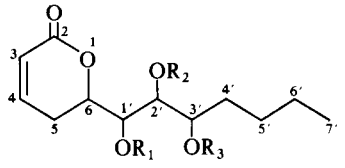
Our search for other minor constituents of *Iboza riparia* resulted in the isolation and structural elucidation of a novel α-pyrone, 1′,2′-dideacetylboronolide (**1**) and of three sterols, sitosterol (**6**), stigmasterol (**8**) and campesterol (**7**). The name of the novel α-pyrone originates from boronolide (**5**) which was previously isolated from *Tetradenia fructicosa*, a plant belonging to the Labiatae [6].

RESULTS AND DISCUSSION

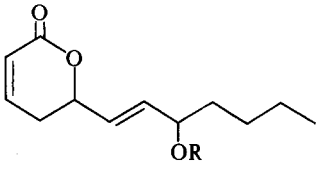
1′,2′-Dideacetylboronolide (**1**), $C_{17}H_{22}O_6$, is a novel α-pyrone isolated in 0.07% yield from the chloroform extract of the leaves of *I. riparia*. The UV (λ_{max}^{EtOH} 212 nm) and IR spectra (ν_{max}^{KBr} 1732 and 1704 cm^{-1}) are consistent with a six-membered α,β-unsaturated lactone moiety, while the presence of hydroxyl functions was also established by the IR spectrum (ν_{OH} 3600–3200 cm^{-1}, broad). The 360 MHz ^1H NMR spectrum ($CDCl_3$) showed an acetoxy group at δ 2.10 (3 H, *s*) and resonances at 0.90 (3 H, *t*, J = 6.75 Hz, Me), 1.32 [4 H, *m*, $(CH_2)_2$] and 1.66 (2 H, *m*, CH_2) which can be attributed to a *n*-butyl group. The characteristic multiplets at 6.03 (1 H, $d \times d \times d$) and 6.95 (1 H, $d \times d \times d$) correspond to the olefinic protons, which display couplings with the non-equivalent allylic protons at the 5-position. The latter resonate at 2.60 and 2.54. The spin pattern at 360 MHz showed practically full first-order characteristics, a phenomenon which was also observed for the remaining spin systems consisting of the four methine units linked to oxygen (see below). The remaining structural units in the molecule point to three oxygen functionalities (two hydroxyl groups and one acetoxy group) at the first three carbons of the heptyl side-chain. The exact position of these three functions was established by NMR analysis, which proved the acetoxy group to be at the 3-position of the side-chain. The methine hydrogen at the 3-position of the side-chain resonated at δ 5.02, while the hydrogen at the 6-position of the ring displayed an absorption at 4.49, both revealing the characteristic splitting pattern required for the proposed structure. The splitting pattern of the hydrogens at the 1′- and 2′-position of the side-chain gave rise to δ values of 3.80 and 3.91 ppm, respectively. All these assignments were unambiguously supported by double irradiation experiments on all protons but those of the *n*-butyl group. Additional evidence for the structure was found in one run of the ^1H NMR spectrum ($CDCl_3$) in which the coupling with the protons of the hydroxyl group (^3J) with the adjacent methine protons was not observed, presumably because of fast exchange of the hydroxyl protons (with traces of water). This established unambiguously the positions of the hydroxyl groups in the side-chain. Additionally, computer-assisted simulation of the spin systems fully supported the correct analysis.

The ^{13}C NMR spectrum of dideacetylboronolide (**1**) is in full agreement with the proposed structure. The carbonyl region showed two peaks at δ 171.6 (acetate

§To whom correspondence should be addressed.



1 $R_1 = R_2 = H$; $R_3 = Ac$
3 $R_1 = R_2 = R_3 = H$
5 $R_1 = R_2 = R_3 = Ac$



2 R = Ac
4 R = H

carbonyl) and 163.4, while the olefinic carbons are situated at 145.4 (*d*; C-4 ring) and 121.1 (*d*; C-3 ring). The aliphatic high-field part of the spectrum revealed six resonances, of which two were quadruplets and four were triplets. The quadruplets represented the methyl groups, namely the terminal Me (13.74) and the acetate methyl (20.97). The four methylene triplets at 22.2, 25.46, 27.2 and 30.3 are attributed to the carbons at C-6′, C-5-ring, C-5′ and C-4′, respectively. It should be noticed that these values correspond very well with calculated values on the basis of shift increment rules. The assignments were independently confirmed by the residual splittings measured in a series of sford (single frequency off-resonance decoupled) spectra, which allowed correlation with ^1H assignments. The remaining four carbons connected to oxygen gave resonances in the 70–80 ppm region, but one resonance line was covered by the peaks of $CHCl_3$. In this case, predicted values based on shift increment rules did not fit, making assignments difficult. Most probably the carbons connected to the hydroxyl groups give rise to peaks at δ 70.22 and 72.07, because the protons linked to them resonate upfield in the ^1H spectrum, thus revealing the lowest and almost equal residual coupling J_R. The chemical shifts at δ 75.56 and 77.12 are then attributed to the C-3′ carbon of the chain and the C-6 carbon of the ring, as they show a larger J_R. The mass spectrum of dideacetylboronolide is characterized by the molecular ion (M^+ at *m/z* 286, 0.8%) besides a peak corresponding to a fragment formed by loss of OH from the parent molecule. The most intense peak is the acetyl fragment (*m/z* 43, 100%) and the majority of the fragment ions could be explained by simple fragmentation patterns. Besides the new α-pyrone (**1**), we also isolated a sterol fraction (0.07%), which could be separated in its components by gas chromatography. By comparison with authentic samples, it was shown that the mixture consisted of sitosterol (65%), campesterol (5%) and stigmasterol (30%). The identification of these sterols was supported by spectrometric data (NMR, MS and IR) and by comparison with reported data [7]. It should be pointed out that sitosterol was recently identified in *I. riparia*, but the isolation of the other sterols, campesterol and stigmasterol, was not reported [4].

EXPERIMENTAL

The plant material was collected in Rwanda and identified as previously described [5].

Isolation. Air-dried leaves of *I. riparia* (Hochst) N.E. Br. (1 kg) were ground to a fine powder which was extracted in a percolator with 15 l. $CHCl_3$. The extract was filtered and evapd *in vacuo* at 40° (135 g). The extract (18.0 g) was chromatographed on a Si gel column in C_6H_6 (600 g with a C_6H_6–$CHCl_3$–MeOH gradient). The fractions eluted with C_6H_6–$CHCl_3$ (25:75) yielded upon evaporation 0.095 g of a mixture of sterols (0.07%). Crystallization from Me_2CO yielded small colourless crystals, mp 136–137°. The mixture of sterols was identified by GLC analysis on UCC W-982, Chromosorb W 80–100, 1.80 m, which revealed it to be a mixture of sitosterol (65%), stigmasterol (30%) and campesterol (5%) by comparison with authentic samples. Additionally, the MS of the mixture of sterols (direct inlet system) exhibited *m/z* values corresponding to the three molecular ions of the sterols. Other fragment ions corresponded satisfactorily with the fragmentation pattern of authentic samples, while the NMR and IR spectral data fully supported the structural determination.

The fractions eluted with $CHCl_3$–MeOH (93:7) yielded upon evaporation 3.8 g of a mixture which was further sepd by prep. TLC (Merck, Si gel $60F_{254}$, 2-mm thickness) with C_6H_6–Et_2O (1:9). The TLC band R_f 0.2 afforded 0.1 g 1,2-dideacetylboronolide (**1**) (0.07%). Crystallization from cyclohexane yielded small colourless needles, mp 80.5–81.0°.

^1H NMR (360 MHz, $CDCl_3$): δ 2.10 (3 H, *s*, MeCO); 6.03 (1 H, *d* × *d* × *d*, $J_{3,4} = 9.7$ Hz, $J_{3,5A} = 0.8$ Hz, $J_{3,5B} = 3.0$ Hz, H-3); 6.95 (1 H, *d* × *d* × *d*, $J_{3,4} = 9.7$ Hz, $J_{4,5A} = 6.1$ Hz, $J_{4,5B} = 2.3$ Hz, H-4); 2.60 and 2.54 (2 H, H_a-5 and H_b-5, respectively, $J_{5A,6} = 3.8$ Hz, $J_{5A,3} = 0.8$ Hz, $J_{5A,4} = 6.1$ Hz, $J_{5B,6} = 11.8$ Hz, $J_{5B,3} = 3.0$ Hz, $J_{5B,4} = 2.3$ Hz, J_{5A}, $J_{5B} = 18.6$ Hz); 0.90 (3 H, *t*, H_3-7′); 1.32 (4 H, *m*, 2 × CH_2); 1.66 (2 H, *m*, H_2-4′); 3.80 (1 H, *d* × *d* × *d*, $J_{1',2'} = 1.9$ Hz, $J_{1',6} = 7.0$ Hz, $^3J_{1',OH} = 7.7$ Hz, H-1′); 4.49 (1 H, *d* × *d* × *d*, $J_{6,1'} = 7.0$ Hz, $J_{6,5A} = 3.8$ Hz, $J_{6,5B} = 11.8$ Hz, H-6); 3.91 (1 H, *d* × *d* × *d*, $J_{2',1'} = 1.9$ Hz, $^3J_{2',OH} \sim 6.5$ Hz, $J_{2',3'} = 5.5$ Hz, H-2′); 5.02 (1 H, $J_{3',2'} = 5.5$ Hz, $J_{3',4'} \sim 6.4$ Hz, H-3′); 2.80 (1 H, *d*, $J_{1',OH} = 7.7$ Hz, 1′-OH); 2.87 (1 H, *d*, $J_{2',OH} = 6.5$ Hz, 2′-OH). ^{13}C NMR (50.29 MHz, $CDCl_3$): δ 171.6 (*s*, MeC=O); 163.4 (*s*, C=O); 145.4 (*d*, C-4 ring); 121.1 (*d*, C-3 ring); 13.74 (*q*, terminal CH_3); 20.97 (*q*, CH_3C=O); 22.2 (*t*, C-6′ chain); 27.2 (*t*, C-5′ chain); 30.3 (*t*, C-4′ chain); 25.46 (*t*, C-5 ring); 70.22 (*d*, C-OH); 72.07 (*d*, C-OH); 75.56 and 77.12 (both *d*, C-OAc and C-6 ring). MS (70 eV), *m/z* (rel. int.): 286 $[M]^+$ (0.8), 269 $[M - OH]^+$ (2.5), 227 $[M^+ - OH{-}CH_2{=}C{=}O]^+$ (2), 209 (2), 189 (9), 171 (2.5), 159 (14), 158 (10), 157 (8), 140 (9), 139 (2), 129 (13), 128 (7), 127 (9), 122 (9), 117 (6), 111 (19), 110 (14), 109 (25), 103 (3), 99 (17), 98 (10), 97 (27), 95 (10), 94 (10), 93 (4), 86 (5), 83 (13), 82 (17), 81 (17), 79 (2.5), 78 (4), 73 (18), 71 (6), 70 (6), 69 (17), 68 (12), 67 (6), 66 (5), 61 (10), 57 (12), 55 (20), 53 (5), 43 $[MeC{\equiv}O]^+$ (100), 42 (9), 41 (30), 40 (5), 39 (18).

REFERENCES

1. Van Puyvelde, L. and Kayonga, A. (1975) *Afr. Med.* **14**, 925.
2. Van Puyvelde, L. (1976) *Rapp. Deuxième Coloq. CAMES*, 60.
3. Watt, J. M. and Breyer-Brandwijk, M. G. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, p. 516. Livingstone, Edinburgh.

4. Zelnik, R., Rabenhorst, E., Matida, A. K., Gottlieb, H. E., Lavie, D. and Panizza, S. (1978) *Phytochemistry* **17**, 1795.
5. Van Puyvelde, L., Dubé, S., Uwimana, E., Uwera, C., Domisse, R. A., Esmans, E. L., Van Schoor, O. and Vlietinck, A. J. (1979) *Phytochemistry* **18**, 1215.
6. France, N. C. and Polonsky, J. (1971) *C. R. Acad. Sci. Paris* **273**, 439.
7. Yamaguchi, K. (1970) *Spectral Data of Natural Products.* Elsevier, Amsterdam.