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NEW α-PYRONES FROM *IBOZA RIPARIA*

LUC VAN PUYVELDE*, SERGE DUBE*, EMMANUEL UWIMANA*, CELINE UWERA*,
ROGER A. DOMMISSE†, EDGARD L. ESMANS†, OMER VAN SCHOOR‡ and ARNOLD J. VLIETINCK‡§
*Faculté de Médecine et des Sciences, Université Nationale de Rwanda, Butare, Rwanda;
†Laboratory of Organic Chemistry, RUCA; ‡Department of Pharmaceutical Sciences, UIA, University of Antwerp, Belgium

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Key Word Index—*Iboza riparia*; Labiatae; new α-pyrones; umuravumbolide; boronolide; structural determination.

Abstract—The structures for umuravumbolide, 5,6-dihydro-6-(3-acetoxy-1-heptenyl)-2-pyrone, a new α-pyrone from *Iboza riparia* (Labiatae) and its corresponding deacylated product have been established. Deacetylboronolide was also isolated and identified by different spectroscopic techniques.

INTRODUCTION

In the course of systematic studies on biologically active substances from medicinal plants of Rwanda, significant chemotherapeutic activity was found in the methanolic extract of *Iboza riparia* leaves. *I. riparia* (Hochst) N.E.Br (Labiatae), formerly known as *I. multiflora* (Benth) E. A. Bruce, is widespread throughout central Africa and reputed to possess several medicinal properties [1]. In Rwanda, where it is known under the name Umuravumba, this species is cultivated around almost every house as a remedy against a wide range of illnesses including malaria, diarrhoea and several kinds of fevers and aches [2, 3]. The isolation and structural elucidation of three α-pyrones are reported in this communication.

RESULTS AND DISCUSSION

Umuravumbolide (**1**), $C_{14}H_{20}O_4$, is a new α-pyrone isolated in 0.13% yield from leaf material of *I. riparia*. Its UV (λ_{max}^{EtOH} 210 nm, log ε 1.029) and IR spectra (ν_{max}^{KBr} 1740 cm^{-1}) are in accord with the presence of a 6-membered α,β-unsaturated lactone [4]. The ^1H NMR spectrum (100 MHz, $CDCl_3$) showed an acetoxymethyl group at δ 2.04 (3H, *s*) and a 3-proton triplet at δ 0.9 (3H, *t*, J = 6 Hz), which has been assigned to a terminal Me group adjacent to a methylene group. The 4-proton and 2-proton multiplets centred at δ 1.32 and 1.49 respectively, have been attributed to the 3 methylene groups of the side-chain. The multiplets at δ 6.88 and 6.04 correspond to the β- and α-protons, respectively of the lactone double bond. These protons are coupled to each other (J = 10 Hz) and to the 2 allylic protons centred at δ 2.36 (2H, *m*). The coupling constants of the latter protons are 5 and 3 Hz with the β-proton (δ 6.88) and 2.5 and 1 Hz with the α-proton (δ 6.04), respectively. The four signals in the region between δ 5.26 and 5.85 prove that the side-chain contains unsaturation and are attributed to the double bond protons, the allylic proton (3-position of the side-chain), which is situated on the carbon bearing the acetoxy function, and the allylic proton in the 6-position of the ring. These data are amply supported by the ^{13}C NMR spectrum (25.15 MHz, $CDCl_3$). Table 1 shows the chemical shifts of the different carbon atoms of **1** and the multiplicities of the peaks in an off-resonance decoupling experiment. The peak at δ 69.45 proves that the acetoxy group is situated at the C-3 position of the side-chain whereas the peaks at δ 130.12 and 131.72 incidate that the double bond of the side-chain must be in the C-1–C-2 position. The steric configuration of this double bond is not yet known but it appears from the IR spectrum (ν_{max}^{KBr} 965 cm^{-1}) to be *trans*. All the other assignments are in good agreement with shift increments from tables and model substances found in the literature [5, 6].

It should be noted that a similar unsaturated lactone named hyptolide (**3**), has been isolated from the botanically related plant *Hyptis pectinata* Poit. This compound

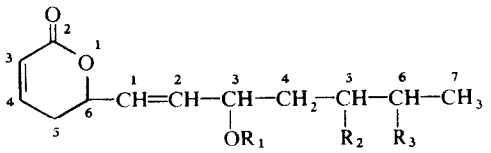
Table 1. ^{13}C NMR data of umuravumbolide (**1**) and deacetylumuravumbolide (**2**)

Carbon atom	δ ($CDCl_3$) (**1**)	δ ($CDCl_3$) (**2**)
C-2	163.37 (*s*)	163.90 (*d*)
C-3	121.73 (*d*)	121.60 (*d*)
C-4	144.15 (*s*)	144.88 (*d*)
C-5	30.09 (*t*)	30.10 (*t*)
C-6	74.07 (*d*)	73.87 (*d*)
C-1 (side-chain)	131.72* (*d*)	127.30 (*d*)
C-2 (side-chain)	130.12* (*d*)	138.20 (*d*)
C-3 (side-chain)	69.45 (*d*)	67.61 (*d*)
C-4 (side-chain)	34.32 (*t*)	36.93 (*t*)
C-5 (side-chain)	27.60 (*t*)	27.60 (*t*)
C-6 (side-chain)	22.47 (*t*)	22.76 (*t*)
C-7 (side-chain)	13.88 (*q*)	14.07 (*q*)

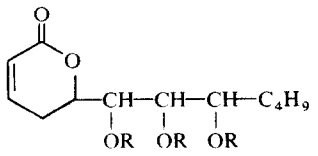
The spectra were recorded on a Jeol JNM-PTF 100 spectrometer operating at 25.15 MHz. Chemical shifts are given in ppm from TMS as internal standard. Multiplicities in an off-resonance experiment are given in parentheses.

* The assignment may be reversed.

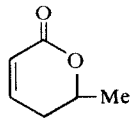
§ To whom inquirics should be made.



1 R_1 = COMe; R_2 = R_3 = H
2 R_1 = R_2 = R_3 = H
3 R_1 = COMe; R_2 = R_3 = OCOMe



4 R = H
5 R = COMe



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has been shown to contain 3 acetoxy functions in the side-chain and its unsaturation was determined by hydrogenation followed by periodate fission and identification of the resulting compounds [7].

Characteristic peaks in the EI-MS of **1** are shown in Fig. 1. No M^+ could be detected under EI conditions at 70 eV. The highest detectable ion was found at *m/e* 210 (12%), which is formed by loss of a neutral ketene molecule. Loss of the α,β-unsaturated lactone group accounts for the peak at *m/e* 155 (54%), whereas the lactone group is indicated by peaks at *m/e* 68 (91%) and 97 (65%). Further fragmentation of the *m/e* 210 ion by loss of H_2O and a subsequent loss of the *n*-butyl radical lead to the intense peaks at *m/e* 192 (22%) and 135 (84%). The presence of the acetyl group could be readily confirmed by a strong peak at *m/e* 43, which is the base peak of the MS, whereas the loss of a ketene group from the M^+ followed by the loss of the *n*-butyl radical by fission α to the OH group accounts for the ion at *m/e* 153 (65%). From the available data, the structure for umuravumbolide was determined as 5,6-dihydro-6-(-3-acetoxy-1-heptenyl)-2-pyrone (**1**).

A second compound was isolated in 0.11% yield and identified as deacetylumuravumbolide, 5,6-dihydro-6-(3-hydroxy-1-heptenyl)-2-pyrone (**2**). Elemental analysis, EI and CI-MS (M^+ at *m/e* 210) established the molecular formula of **2** as $C_{12}H_8O_3$. The presence of a free OH function was established by the 3430 and 1055 cm^{-1} bands in the IR and a singlet at δ 2.7 (1H, *s*) in the ^1H NMR spectrum together with the absence of the characteristic peaks of the acetyl function in the different spectra. Diagnostic peaks of **2** were found in the CI-MS (CH_4, probe) at *m/e* 211 $M^+ + 1$ (19%), 210 M^+ (2%), 209 $M^+ - 1$ (7%) and at *m/e* 193 (100%) and 175 (17%), which were formed from the $M^+ + 1$ by loss of 1 and 2 molecules of H_2O, respectively. Characteristic addition-ions were found at *m/e* 221 $M^+ - H_2O + C_2H_5$ (18%) at *m/e* 233 ($M^+ - H_2O + C_3H_5$] (12%).

A third new α-pyrone was isolated in 0.06% yield and shown to be deacetylboronolide, 5,6-dihydro-6-(1,2,3-trihydroxyheptyl)-2-pyrone (**4**), having the molecular formula $C_{12}H_{20}O_5$ as was revealed by elemental analysis, EI- and CI-MS. The corresponding triacetyl derivative, which was named boronolide (**5**), was previously isolated by Franca and Polonsky [7] from *Tetradenia fructicosa*, a plant which also belongs to the Labiatae. The ^1H and ^{13}C NMR chemical shift data of **5** (100 and 25.15 MHz, $CDCl_3$) amply confirm the proposed structure (see Experimental). No M^+ for **4** could be detected in the MS under EI conditions at 70 eV, but by CI-MS a parent ion was observed at *m/e* 244 (2%). The highest detectable ion under EI conditions was found at *m/e* 187 (4%) and was formed from the M^+ by loss of the *n*-butyl radical. Other characteristic fragmentations generated by simple or multiple bond scissions are presented in Fig. 2. Since in such polyhydroxylated compounds thermal decomposition often occurs, which leads to complicated MS, **4** was converted into its TMSi-derivative by treatment with BSTFA, and CI- and EI-MS of this TMSi-derivative were recorded. The diagnostic peaks of the EI-MS are depicted in Fig. 3. Characteristic peaks of the TMSi-derivative of **4** in the CI-MS (CH_4, probe) were found at *m/e* 389 $M^+ + 1$ (20%) and 373 (20%), 299 (49%) and 209 (100%), which were formed from the $M^+ + 1$ by loss of CH_4, TMSiOH and 2× TMSiOH, respectively. Loss of CH_4 and H_2O from the ion *m/e* 299 accounts for the peaks *m/e* 283 (33%) and 281 (18%), respectively. Finally, small addition-ions were found at *m/e* 417 $M^+ + C_2H_5$ (2%) and 429 $M^+ + C_3H_5$ (1.5%). The stereochemistry of C-1 of the side-chain of boronolide is reported as *R* [8], whereas the stereochemistry of the portion containing the OH groups was not determined. Preliminary GC–MS experiments in our laboratory have shown that the TMSi-derivative of **4** was a mixture of at least 3 different stereoisomers.

We are currently investigating the potential chemotherapeutical and pharmacological activities of the

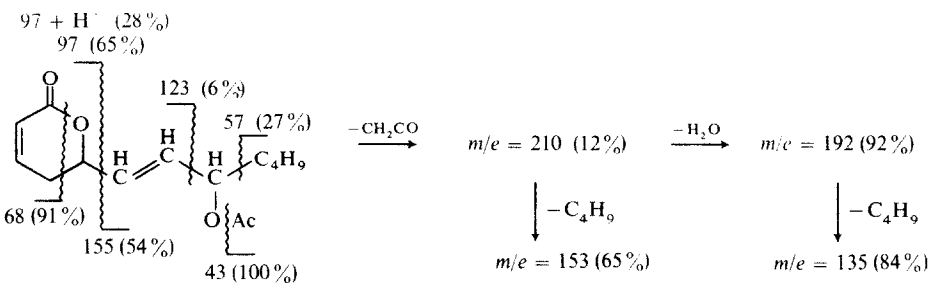

Fig. 1. Diagnostic mass fragmentations of umuravumbolide (EI conditions).

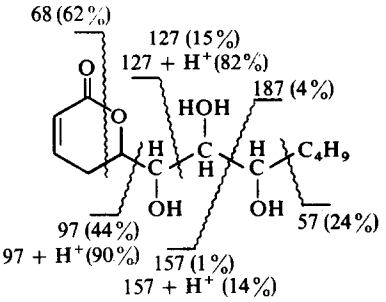

Fig. 2. Diagnostic mass fragmentations of deacetylboronolide (EI conditions).

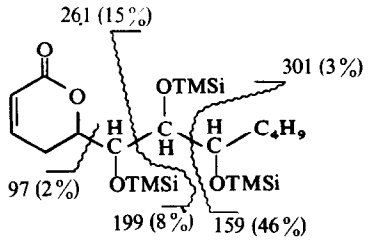

Fig. 3. Diagnostic mass fragmentations of TMSi-deactylboronolide (EI conditions).

isolated α,β-unsaturated lactones. However, it has recently been reported that injection of the biogenetically related parasorbic acid (**6**) in rats and mice leads to the development of tumors [9]. Although not proven as yet, all α,β-unsaturated lactones should be considered as potential carcinogens [10].

EXPERIMENTAL

Both low and high resolution EI-MS (probe) were recorded at 70 eV. CI-MS (CH_4, probe) were recorded by Dr. M. Claeys, University of Antwerp, Belgium, at 150 eV. Microanalyses were performed by Mr. J. Tamas, University of Sherbrooke, Canada.

Plant material. I. riparia was collected in October 1976 in the 'commune' Huye, Rwanda. Identification was performed by Dr. G. Troupin, Musée Royale de l'Afrique Centrale, Tervuren, Belgium and by Mr. R. Barabwiliza, I.N.R.S., Butare, Rwanda. Voucher specimens are deposited in the Herbaria of I.N.R.S., Butare and the Nationale Plantentuin van België, Meise, Belgium.

Isolation. Air-dried leaves of *I. riparia* (1 kg) were ground to a fine powder, which was extracted with MeOH (Soxhlet) for *ca* 40 hr. The extract was filtered and conc *in vacuo* at 40°, then suspended in 2% citric acid (1.2 l.) and filtered after stirring at room temp. for *ca* 12 hr. The suspension was filtered, defatted with petrol, (5 × 600 ml) and extracted with $CHCl_3$ (5 × 300 ml). The $CHCl_3$ phase was evapd to a brown oil (6.8 g), which was chromatographed on a Si gel column in C_6H_6 (320 g with a C_6H_6–$CHCl_3$–MeOH gradient). The fractions eluted with $CHCl_3$ yielded upon evapn 1.3 g compound **1**, which was obtained as a colourless oil and which could not be crystallized at room temp. However, on standing for 18 hr at 0°, small white crystals were formed. TLC on Si gel F_{254}, C_6H_6–Et_2O (1:9) showed a single spot, R_f 0.7. With modified Dragendorff (Munier) and $SbCl_3$ spray reagents a colour reaction was also obtained. $[\alpha]_D^{25}$ 0° (EtOH, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 210 (1.029). IR ν_{max}^{KBr} cm^{-1}: 1740 (ester carbonyl), 1720 (α,β-unsaturated δ-lactone), 1685 (sh), 965 (alkene, *trans*), 1245 and 1035 (acetate). ^1H NMR (100 MHz, $CDCl_3$): δ 0.9 (3H, *t*, J = 6 Hz, H-7 side-chain), 1.08–1.43 (4H, *m*, H-6 and H-5 side-chain), 1.49 (2H, *m*, H-4 side-chain), 2.04 (3H, *s*, acetyl), 2.36 (2H, *m*, H-5,5′ ring), 5.26–5.85 (4H, *m*, H-3, H-2, H-1 side-chain and H-6 ring), 6.04 (1H, *m*, $J_{3,4}$ = 10 Hz, $J_{3,5}$ = 2.5 Hz, $J_{3,5'}$ = 1 Hz, H-3 ring), 6.88 (1H, *m*, $J_{4,3}$ = 10 Hz, $J_{4,5}$ = 5 Hz, $J_{4,5'}$ = 3 Hz, H-4 ring). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 13.88 (*q*, C-7 side-chain), 22.47 (*t*, C-6 side-chain), 27.60 (*t*, C-5 side-chain), 30.09 (*t*, C-5 ring), 34.32 (*t*, C-4 side-chain), 69.45 (*d*, C-3 side-chain), 74.07 (*d*, C-6 ring), 121.73 (*d*, C-3 ring), 130.12 (*d*, C-2 side-chain), 131.72 (*d*, C-1 side-chain), 144.15 (*d*, C-4 ring), 163.37 (*s*, C-2 ring). EI-MS 70 eV *m/e* (rel. int.): 210 [M^+ − 42] (12%), 192 (22), 155 (54), 153 (65), 135 (84), 123 (6), 98 (28), 97 (65), 68 (91), 57 (27), 43 (100). (Found: C, 66.70; H, 8.05. $C_{14}H_{20}O_4$ requires: C, 66.73; H, 8.00%).

The fractions eluted with $CHCl_3$–MeOH (99:1) afforded upon evapn 1.15 g compound **2**. Crystallization from cyclohexane yielded colourless needles, mp 45.5–46.0. R_f 0.5, $[\alpha]^{25}$ 0° (EtOH, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 205 (1.025). IR ν_{max}^{KBr} cm^{-1}: 3430, 1055 (α,β-unsaturated sec. alcohol), 1715 (α,β-unsaturated δ-lactone), 1680, 965 (alkene. *trans*). ^1H NMR (100 MHz, $CDCl_3$): δ 0.88 (3H, *t*, J = 6 Hz, H-7 side-chain), 1.32 (6H, *m*, H-6, H-5 and H-4 side-chain), 2.36 (2H, *m*, H-5, 5′ ring), 2.70 (1H, *s*, —OH), 4.33 (1H, *m*, H-4 side-chain), 5.28 (1H, *m*, H-6 ring), 5.52 (2H, *m*, H-2 and H-1), 5.92 (1H, *m*, $J_{3,4}$ = 9 Hz, $J_{3,5}$ = $J_{3,5'}$ = 2 Hz, H-3 ring), 6.78 (1H, *m*, $J_{4,3}$ = 9 Hz, $J_{4,5}$ = $J_{4,5'}$ = 4 Hz, H-4 ring). ^{13}C NMR (25.15 MHz, $CDCl_3$): δ 14.07 (*q*, C-7 side-chain), 22.76 (*t*, C-6 side-chain), 27.6 (*t*, C-5 side-chain), 30.1 (*t*, C-5 ring), 36.93 (*t*, C-4 side-chain), 67.61 (*d*, C-3 side-chain), 73.87 (*d*, C-6 ring), 121.6 (*d*, C-3 ring), 127.3 (*d*, C-1 side-chain), 138.2 (*d*, C-2 side-chain), 144.88 (*d*, C-4 ring), 163.9 (*s*, C-2 ring). MS 70 eV *m/e* (rel. int.): 210 [M^+] (2), 192 (4), 153 (60), 135 (100), 113 (7), 98 (16), 97 (13), 68 (33), 57 (33). CI-MS (CH_4, probe) *m/e* (rel. int.): 211 [$M^+ + 1$] (19), 210 [M^+] (2), 209 [$M^+ - 1$] (7), 193 ($M^+ + 1 - H_2O$] (100), 175 [$M^+ + 1 - 2H_2O$] (17), 221 [$M^+ - H_2O + C_2H_5$] (18), 233 [$M^+ - H_2O + C_3H_5$] (12%). (Found: C, 68.82; H, 8.54. $C_{12}H_{18}O_3$ requires: C, 68.64; H, 8.64%).

The fractions eluted with $CHCl_3$–MeOH (19:1) yielded upon evapn 616 mg compound **4**. Recrystallization from C_6H_6–petrol (2:3) afforded colourless prisms, mp 105.5–106°, R_f 0.1, $[\alpha]_D^{25}$ 0° (EtOH, *c* 1,0). UV λ_{max}^{EtOH} nm (log ε): 205 (0.977). IR ν_{max}^{KBr} cm^{-1}: 3450, 3260, 1080 (sec. alcohol), 1710 (α,β-unsaturated δ-lactone). ^1H NMR (100 MHz, $CDCl_3$): δ 0.93 (3H, *t*, J = 6 Hz, H-7 side-chain), 1.41 (6H, *m*, H-6, H-5 and H-4 side-chain), 2.67 (2H, *m*, H-5 and H-5′ ring), 3.4 (3H, *brs*, —OH), 3.81 (3H, *m*, H-3, H-2, H-1 side-chain), 4.6 (1H, *m*, H-6 ring), 6.11 (1H, *m*, H-3 ring), 7.05 (1H, *m*, H-4 ring). ^{13}C NMR (21.15 MHz, $CDCl_3$): δ 14.03 (*q*, C-7 side-chain), 22.71 (*t*, C-6 side-chain), 25.82 (*t*, C-5 ring), 27.81 (*t*, C-5 side-chain), 38.44 (*t*, C-4 side-chain), 70.57, 74.16, 77.37 and x (*d*, C-3, C-2, C-1 side-chain and C-6 ring: unassigned because the exact position of one of those cannot be determined by overlapping with the signals of the solvent), 120.95 (*d*, C-3 ring), 146.24 (*d*, C-4 ring), 164.15 (*s*, C-2 ring). EI-MS 70 eV (rel. int.): 187 [$M^+ - 57$] (4), 158 (14), 157 (1), 128 (82), 127 (15), 98 (90), 97 (44), 73 (100), 68 (62), 57 (24). CI-MS (CH_4, probe) *m/e* (rel. int.): 245 [$M^+ + 1$] (98), 244 [M^+] (2), 243 [$M^+ - 1$] (7), 227 [M^+) $1 - H_2O$] (39), 209 [$M^+ + 1 - 2H_2O$] (100), 191 [$M^+ + 1 - 3H_2O$] (38), 273 [$M^+ + C_2H_5$], 285 [$M^+ + C_3H_5$]. EI-MS TMSi-derivative, 70 eV (rel. int.): 301 [$M^+ - 87$] (3), 261 (15), 199 (8), 159 (46), 97 (2). CI-MS TMSi-derivative (CH_4, GC–MS)

m/e: 389 $[M^+ + 1]$ (20), 388 $[M^+]$ (1%), 387 $[M^+ - 1]$ (1), 373 $[M^+ + 1 - CH_4]$ (20), 317 $[M^+ - 72]$ (48), 299 $[M^+ + 1 - TMSiOH]$ (49), 283 (299 $- CH_4$) (33), 281 (299 $- H_2O$) (18), 209 $[M^+ + 1 - 2 \times TMSiOH]$ (100), 417 $[M^+ + C_2H_5]$ (2), 429 $[M^+ + C_3H_5]$ (1, 5).

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