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A BUTENOLIDE ATYPICAL OF THE RANUNCULACEAE: AQUILEGIOLIDE FROM *AQUILEGIA ATRATA* (VAR. *ATROVIOLACEA*)

ANTONIO GUERRIERO and FRANCESCO PIETRA

Istituto di Chimica, Università di Trento, 38050 Povo-Trento, Italy

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Key Word Index—*Aquilegia atrata*, Ranunculaceae, butenolides

Abstract—Roots of *Aquilegia atrata* have afforded 6β-hydroxy-2(6,7-dihydro-7aβH)-benzofuranone (aquilegiolide) and its 7aαH-isomer, or their enantiomers, two butenolides atypical of the Ranunculaceae Hot aqueous 20% sulphuric acid rapidly equilibrates the two isomers in a 1 4 ratio

INTRODUCTION

Ranunculaceae have afforded some C_5 α,β-unsaturated lactones [1, 2] among which, however, only protoanemonin (**1**) [3, 4] is not an artifact of the extraction process [5]

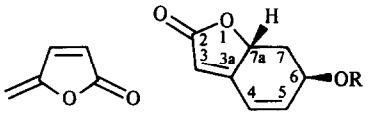
It is interesting, therefore, to have now found in *Aquilegia atrata*, var *atroviolacea*, of the Trentino area, two ring-fused butenolides (**2** and **4** or their enantiomers), which are atypical of the Ranunculaceae

One of these butenolides (**4**) has already been described (but this needs some correction here) as the only butenolide from acid hydrolysis of menisdaurin, a nitrile glucoside isolated from *Menispermum dauricum* (Menispermaceae) [6]

RESULTS AND DISCUSSION

Roots were lyophilized and extracted first with ethyl ether and then with methanol at reflux The residue from solvent evaporation was column chromatographed at medium pressure on silica gel with ethyl ether, whereby little pure **2** and **4** were obtained from the first and the last fraction, respectively Efficient separation of **2** and **4** was then completed by reverse phase HPLC

Compound **2** (0 01% in fresh roots) was obtained as colourless needles The conjugated chromophore is suggested by the 253 nm absorption, while the IR spectrum clearly shows hydroxyl, conjugated lactone carbonyl, and olefine absorptions The high-resolution mass spectrum revealed the elemental composition of the molecular ion, whilst linked scans showed the loss, from the molecular ion, of water, formyl, ketene and carbon dioxide and, from $[M-CHO]^+$, of carbon monoxide The fragmentation pattern is clearly in accordance with structure **2**, which is further supported by MS deuteriation experiments with deuteriated methanol These showed incorporation of one deuterium in all ions except *m/z* 134, clearly in accordance with alcoholic hydrogen exchange for an hydroxyl group at either C-6 or C-7 In fact, loss of either water or of DHO from the molecular ion introduces a $\Delta^{6,7}$ double bond Moreover, all carbon resonances, with the expected multiplicities, could be observed for **2** Finally, the



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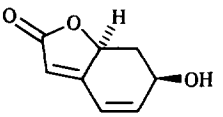
3 R = Ac



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^{1}H NMR spectrum fully supports structure **2** with the relative stereochemistry shown

Acetylation of **2** gave **3** which showed the expected spectra Compound **4** (0 04% in fresh roots) was also obtained as colourless needles The UV and mass spectra were quite similar to those for **2** Also, both the ^{13}C NMR and ^{1}H NMR spectra, as well as the rotatory power, were practically identical with those published for menisdaurilide [6] This has been reported as the only butenolide from hydrolysis, in hot 20% sulphuric acid, of menisdaurin, a nitrile glucoside isolated from *Menisperum dauricum* [6] This report [6] surprised us as we had observed that **2** and **4** equilibrate to a 1 4 mixture in hot 20% sulphuric acid in time intervals comparable to those for hydrolysis of menisdaurin In fact, in our hands, menisdaurin, which gave a single HPLC peak, hydrolysed, under the previously reported conditions [6], to give a 1 4 mixture of, respectively, **2** and **4** Also, equilibration of **2** and **4** at room temperature in either organic solvents, or water, or 20% sulphuric acid was extremely slow, though the process was faster when starting from **2** than from **4**

The absolute configuration of **4** has been tentatively inferred from CD and ORD spectra [6] However, because of the unreliability of such conclusions [6], we have attempted, albeit unsuccessfully, a chemical route Thus, attempted furanization of **2** with DIBAL, as a prelude to ozonization to get malic acid, only gave a weak Ehrlich-reactive TLC spot, possibly due to benzo[b]furan, while all **2** disappeared DIBAL reaction on the ester of **2** with a bulky moiety, such as (−)-menthyl chloroformate [7], was equally unsuccessful Finally, with both **2** and **4** also the Horeau method failed, the optimal yield being less than 2% In conclusion, the absolute configuration of **2** (and **4**) remains to be established

EXPERIMENTAL

Prep, medium pressure liquid chromatography was carried out on a Jobin-Yvon Miniprep apparatus with LiChroprep SI 60, 15–25 μm, 50 g Prep and analytical HPLC was carried out on a Perkin-Elmer Series 3B apparatus with either a Silica A, 10 μm, 0 26 × 25 cm P E column, or for, reverse phase, with LiChrosorb RP-18, 7 μm, 0 4 × 25 cm and 1 × 25 cm Merck columns In all cases, UV monitoring was carried out with a Jasco Uvidec 100-III detector Merck Kieselgel 60 F_{254} plates were used for TLC

^{1}H NMR spectra were taken with a Varian CFT 20 spectrometer modified for ^{1}H (80 MHz) spectra and equipped with a microprobe for ^{13}C (20 MHz) spectra Chemical shift are given with respect to TMS as an int standard ^{13}C NMR multiplicities are from off-resonance spectra MS were carried out with a VG ZAB2F mass spectrometer at 70 eV

Extraction Roots (0 9 kg, collected at Fai della Paganella, Trento, in June 1980 and June 1981) were first lyophilized and then homogenized in a blender without added solvent The homogenate was then Soxhlet extracted, first with Et_2O and then with MeOH, for 12 hr During this time the solvent was renewed three times, in order to avoid prolonged heating of the compounds (Approximately 3/4 of **2** + **4** were extracted by Et_2O and 1/4 by MeOH) The same results were obtained on extraction of roots with EtOH at room temp

Isolation The combined Et_2O and MeOH extracts were evaporated to dryness *in vacuo* and the residue was chromatographed on the Jobin-Yvon app with Et_2O Heads and tails gave pure **2** and **4**, respectively Central fractions were evaporated, one at a time, and then subjected to reverse phase HPLC, MeCN–H_2O (4 96), whereby **4** and **2** were eluted at 12 and 14 min, respectively Compounds **2** and **4** could also be separated from one another by HPLC on the Silica A column, *n*-hexane–*iso*-PrOH (8 2)

6β-Hydroxy-2(6,7-dihydro-7aβH)-benzofuranone (**2**) Colourless needles, mp 96–98° (C_6H_6), $[\alpha]_D^{25}$ −419 6° (MeOH, *c* 0 6), IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3680, 3610, 3450, 1745, 1650, UV λ_{max}^{MeOH} nm (log ε) 253 (4 29), EIMS *m/z* (rel int) 152 (152 0491 ± 0 002, calc for $C_8H_8O_3$ 152 047)$[M]^+$ (66), 134 $[M-H_2O]^+$ (19), 123 $[M-CHO]^+$ (62), 110 $[M-COCH_2]^+$ (100), 108 $[M-CO_2]^+$ (27), 107 $[108-H]^+$ (36), 106 $[108-H_2]^+$ (46), 95 $[123-CO]^+$ (93), ^{1}H NMR [$(CD_3)_2CO$] δ6 65 (1H, *d*, $J_{4,5}$ = 9 7 *Hz*, *H*-4), 6 37 (1*H*, *br dd*, $J_{5\,4}$ = 9 7, $J_{5\,6}$ = 4 5 Hz, H-5), 5 82 (1H, *br s*, H-3), 5 27 (1H, *ddd*, $J_{7a,7ax}$ = 12 6, $J_{7a\ 7eq}$ = 5 2, $J_{7a,3}$ = 1 9 Hz, H-7a), 4 60 (1H, *ddd*, $J_{6,5}$ = 4 5, $J_{6,7eq}$ = 1 9, $J_{6,7ax}$ = 4 1 Hz, H-6), 3 0 (1H, *br*, OH, exchangeable with D_2O), 2 51 (1H, *br dd*, J_{gem} = 12 6, $J_{7\beta,7a}$ = 5 2 Hz, H-7β), 1 73 (1H, *ddd*, J_{gem} = 12 6, $J_{7\alpha\ 7a}$ = 12 6, $J_{7\alpha,6}$ = 4 1 Hz, H-7α), ^{13}C NMR [$(CD_3)_2CO$] δ 173 7 (*s*, C-2), 164 4 (*s*, C-3a), 139 6 (*d*, C-3), 121 4 (*d*, C-5), 112 2 (*d*, C-4), 77 0 (*d*, C-7a), 64 3 (*d*, C-6), 38 0 (*t*, C-7) Quite significant were the following irradiations (a) at δ5 82, whereby the δ5 27 *ddd* became a *dd* with *J* 12 6, 5 2 Hz, (b) at δ5 27, whereby the δ5 82 *br s* became an *s*, while the δ2 51 *br dd* became a *br d* with *J* = 12 6 Hz, and the δ1 73 *ddd* became a *dd* with *J* = 12 6, 4 1 Hz, (c) at δ4 60, whereby the δ6 37 *br dd* became a *br d* with *J* = 9 7 Hz, while the δ2 51 *br dd* became a *dd* with *J* = 12 6, 5 2 Hz and the δ1 73 *ddd* became a *dd* with *J* = 12 6, 12 6 Hz, (d) at δ2 51, whereby, other than the inverse phenomenon already described above for irradiation at either δ5 27 and 4 60, we noticed long range couplings of H-7β with both H-3 (the δ5 82 *br s* became a *d* with *J* = 1 9) and H-5 (the δ6 37 *br dd* became a sharp *dd*), while the δ1 73 *ddd* became a *dd* with *J* = 12 6, 4 1 Hz

6β-Acetoxy-2(6,7-dihydro-7aβH-benzofuranone (**3**) Obtained on standard acetylation (Ac_2O–pyridine, room temp) of **2** EIMS *m/z* (rel int) 194 $[M]^+$ (10), 152 $[M-COCH_2]^+$ (100), 134 $[M-AcOH]^+$ (23), ^{1}H NMR ($CDCl_3$) δ6 72 (1H, *d*, *J* = 9 7 Hz, H-4), 6 30 (1H, *br dd*, *J* = 4 7, 4 5 Hz, H-5), 5 87 (1H, *br s*, H-3), 5 58 (1H, *ddd*, *J* = 4 5, 4 0, 1 9 Hz, H-6), 5 20 (1H, *ddd*, *J* = 12 6, 5 2, 1 9 Hz, H-7aβ), 2 63 (1H, *br dd*, *J* = 12 6, 5 2 Hz, H-7β), 2 09 (3H, *s*, Me), 1 86 (1H, *ddd*, *J* = 12 6, 12 6, 4 0 Hz, H-7α)

Isomerization of **2** *and* **4** When pure **2** was left in the solvent mixture used for HPLC, slow isomerization to **4** occurred After 2 days, the **2** **4** ratio was 12 1 in the *n*-hexane–*iso*-PrOH (8 2) mixture, and 10 1 in the MeCN–H_2O (4 96) mixture When pure **4** was left as above for the same time, the **4** **2** ratio was higher than 20 1 in both solvent mixtures When either pure **2** or pure **4** were left in 20% aq H_2SO_4 at 100° for 3 hr, a **2**/**4** = 1 4 mixture resulted The same **2**/**4** ratio was obtained when solns in water of either pure **2** or pure **4** were first made alkaline with KOH and then acidified at room temp When either pure **2** or pure **4** were stored as crystals at −20°, the isomerization process started to become detectable only after several months

Hydrolysis of menisdaurin Menisdaurin (*ca* 0 5 mg) was heated in 1 ml of 20% aq H_2SO_4 at 100° for 3 hr The mixture was cooled and then extracted with EtOAc according to the original procedure [6] The Et_2O extract was evaporated at red pres and HPLC analysed to give **2**/**4** = 1 4

A specimen from our collection of *A atrata* (var *atroviolacea*) has been deposited in the European Herbarium at the British Museum (Natural History), London

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1-(3,4-DIHYDROXY-5-METHOXYPHENYL)-3-METHYLBUT-2-ENE FROM THE LIVERWORT *PLAGIOCHILA RUTILANS*

SIEGFRIED HUNECK,* JOSEPH D CONNOLLY,† LESLIE J HARRISON,† ROBERT S I JOSEPH† and THOMAS POCS‡

*Institute of Plant Biochemistry, Research Centre for Molecular Biology and Medicine of the Academy of Sciences of the G D R, G D R -401 Halle/Saale, Weinberg, German Democratic Republic, †Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U K, ‡Research Institute for Botany of the Hungarian Academy of Sciences, 2163 Vácrátót, Hungary

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Key Word Index—*Plagiochila rutilans*, Hepaticae, 1-(3,4-dihydroxy-5-methoxyphenyl)-3-methylbut-2-ene

Abstract—Extraction of the liverwort *Plagiochila rutilans* afforded 1-(3,4-dihydroxy-5-methoxyphenyl)-3-methylbut-2-ene, the structure of which was confirmed by synthesis

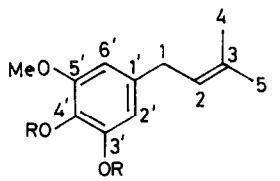
INTRODUCTION

The Cuban liverwort *Plagiochila rutilans* Lindb of the family Plagiochilaceae (Joerg) M Mull is characterized by its peppermint-like odour Chromatography of the diethyl ether extract of the liverwort, collected by one of us (T P) in Cuba, yielded, in addition to an oil with a peppermint-like odour, a crystalline compound which was shown to be 1-(3,4-dihydroxy-5-methoxyphenyl)-3-methylbut-2-ene (**1**) by an examination of its ^{1}H NMR and ^{13}C NMR spectra The structure was confirmed by synthesis

RESULTS AND DISCUSSION

Compound **1** had the formula $C_{12}H_{16}O_3$ (*m/z* 208) and resonances in its ^{1}H NMR spectrum for a dimethylallyl group attached to a benzene ring δ1 7 (*s*, 6H, 2 × vinyl Me), 3 2 (*d*, 2H, Ar–CH_2–) and 5 2 (*t*, 1H, –CH=C<) The trioxygenated nature of the aromatic ring was revealed by the presence of two *meta*-coupled aromatic protons [δ6 20 and 6 29 (both *d*, J = 3 Hz)], a methoxyl group (δ3 80) and two phenolic hydroxyl groups [δ4 7 (*br s*, 2H, exchangeable with D_2O)] The ^{13}C NMR chemical shifts of the oxygenated aromatic carbons (δ148 5, 137 2 and 146 9) clearly indicated that the oxygen functions were attached to positions C-3′, C-4′ and C-5′ while the non-equivalence of the *meta*-coupled aromatic protons required the attachment of the methoxyl group to positions C-3′ or C-5′ These data led uniquely to the structure 1-(3,4-dihydroxy-5-methoxyphenyl)-3-methylbut-2-ene (**1**) for the natural product

This structure was confirmed by synthesis Methyl 3-(3,4,5-trimethoxyphenyl)-propanoate [1], prepared by methylation and hydrogenation of 3,4,5-trimethoxycinnamic acid, was treated with excess methyl magnesium iodide to give the expected alcohol (**2**) Dehydration with



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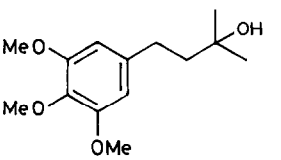
3 R = Me



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