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2-ACYL-4-ALK(EN)YLTETRONIC ACID, A NEW OXIDATION PRODUCT OF HOP α-ACIDS

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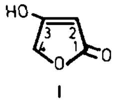
Abstract—Singlet oxygen transforms (−)(*R*)-humulone **2b** and (−)(*R*)-tetrahydrohumulone **2a** into ketene radical intermediates via a radical reaction, which are intramolecularly trapped to give the 2-acyl-4-alkyl- or alkenyl-tetronic acids. These are isolated as a 50:50 mixture of the two enol forms **5** and **6**.

The chemical transformations of the hop bitter acids with retention of their carbocyclic five or six membered carbocyclic ring system are well known.[2] Ring opening of the bitter acids by oxidation, leading to complex mixtures of rather simple compounds, has been studied extensively.[3] It is however conceivable, that more complex, multifunctional open chain compounds are intermediately formed, which possibly could also lead to stable products. There are reasons to believe this; e.g. the fact that isomerized hop extracts contain at the most 70–80% iso-α-acids, the rest being of unknown composition. We report here on a new class of compounds derived from α-acids, which indeed arise by oxidative ring opening followed by intramolecular reactions of the open chain polycarbonyl intermediate.

The new acid is a 2 - acyl - 4 - alk(en)yltetronic acid, which also may be regarded as a 3 - acyl - 5 - alk(en)yl - 2,4 - dihydroxyfuran or as a 2 - acyl - 4 - alk(en)yl - 3 - hydroxybutenolide. Simple furans have been reported to give a "cardboard flavour" to beer, particularly the derivatives of furfural.[4] Dihydro- and tetrahydrofurans occur in hop oil[5] and in oxidation mixtures of hop bitter acids,[6] while 3-ketotetrahydrofurans, partly enolized, constitute a characteristic structural entity of the humulone isomerization products, obtained in alkaline methanol.[7]

Various substituted tetronic acids, which possess the basic structure **1**, are important natural products, such as vitamin C, penicillic acid, mould metabolic products and colouring matters found in lichens.[8]

Tetronic acids were not described before in hop chemistry and, as far as we know, our case is the first example of a natural 2 - acyl - 4 - alk(en)yltetronic acid.



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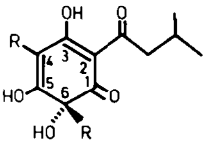
2 - (3′ - *Methyl) butanoyl* - 4 - (3′ - *methyl)butyltetronic acid*

Singlet oxygen attack at C_4 in (−)(*R*)-tetrahydrohumulone **2a** leads, via intermediate **3a**, to tetrahydrohumulinone **4a**, the major reaction product.[9] Counter current distribution with the two phase system ether/0.25 M phosphate buffer pH = 5.6 reveals, after 550 transfers, the presence of a minor product with K = 0.1.

It is a pale yellow optically inactive solid with m.p. 30–31°C. The molecular weight is 254 (absorption maxima at 265 nm in acidic and at 262 and 230 nm in alkaline methanol). In the 300 MHz ^1H NMR spectrum, the signals of the 3-methylbutanoyl and the 3-methylbutyl side chains are easily recognizable in the region below δ 3. An acidic hydrogen atom is found at δ 12.57, but most characteristic is the occurrence of two doublet of doublets at δ 4.61 and δ 4.48, respectively, each approximately accounting for half a proton. These two distinct signals collapse on addition of a trace of triethylamine. It follows that the product consists of a 50:50 mixture of two enol forms, most probably **5a** and **6a**, respectively. These represent in fact the two most stable enol tautomers of 2 - (3′ - methyl)butanoyl - 4 - (3′ - methyl)butyltetronic acid. Individual distinction is possible, since the proton at the α-carbon atom with respect to the carbonyl function in **5a** resonates at higher field compared to the corresponding atom next to the double bond in **6a**.[10] It is normal that tetronic acids are enolized for 100%. Double enolization, which would imply formation of 2,4-dihydroxyfuran systems, such as **7**, does not occur, except in cases of strong stabilization by hydrogen bridges.[11]



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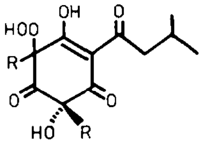

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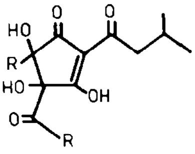

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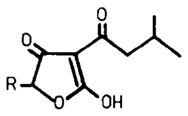

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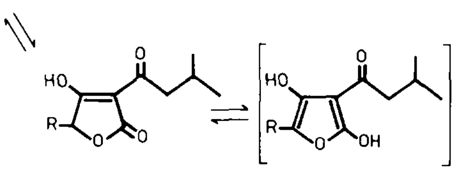


6 7

a: R = $-CH_2CH_2CH(CH_3)_2$
b: R = $-CH_2CH{=}C(CH_3)_2$

2 - (3' - *Methyl)butanoyl* - 4 - (3' - *methyl)but* - 2' - *enyltetronic acid*

The tetronic acid, analogous to **5a** and **6a**, but formed from (−)(R)-humulone (**2b**), was found as a minor product in the isomerization mixture of **2b**, obtained on boiling 15 g in 30 l of buffer pH = 5.4 and without excluding contact with air. It is possible that **2b** itself, which absorbs part of the incident visible light, acts as a sensitizer to produce singlet oxygen. Evidently, the same reaction course just described, can produce the analogous compounds **5b** and **6b**, starting from intermediate **3b**. The yield of the tetronic acids is enhanced when working in oxygen atmosphere.

The product, isolated as the band with K = 0.35 of the counter current distribution in iso-octane/0.25 M buffer pH = 3.95, is a pale yellow oil with a molecular weight of 252 and absorption maxima at 267.5 nm in acidic and at 260 and 227.5 nm in alkaline methanol.

The 300 MHz ^{1}H NMR spectrum shows, besides the signals for the hydrogen atoms of the two side chains, again two doublet of doublets in a 50:50 ratio, collapsing on addition of triethylamine.

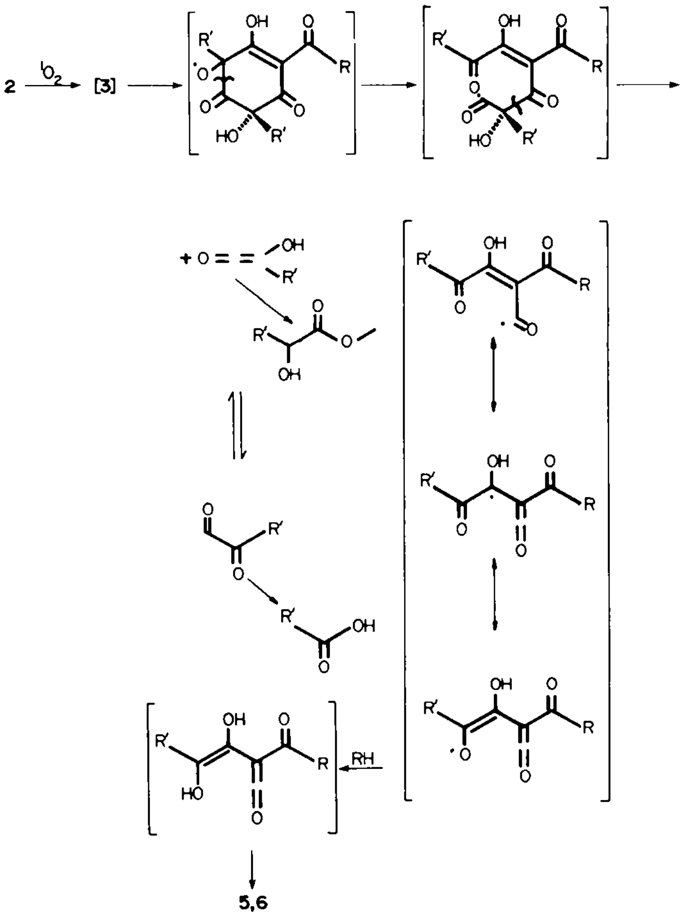
The formation of the tetronic acids **5** and **6** from **2** can be explained by ring cleavage of the primary singlet oxygen reaction product **3**. The reaction sequence probably starts with splitting of the peroxide bond. The radical, thus produced, can stabilize itself by ring cleavage, to be followed by a second cleavage, whereby a ketenol is split off. This could be trapped as methyl 2 - hydroxy - 5 - methylhexanoate, when the singlet oxygen oxidation was performed in methanol, while part of it was found as 4-methylpentanoic acid, formed via tautomerization of the ketenol and subsequent oxidation and oxidative decarboxylation. A hydrogen radical abstraction (either from the hydroperoxide **3**, from the starting material or from the solvent) transforms the main radical fragment into an enediol - acyl - ketene derivative, which cyclizes to give the tetronic acids **5** and **6** (Chart 1).

EXPERIMENTAL

The 300 MHz ^{1}H NMR spectra are obtained on a Varian HR-300 spectrometer with TMS as internal reference, the mass spectra on a CEC 21-104 instrument, the UV spectra on a Cary 15 spectrophotometer and the high resolution MS accurate mass measurements on an AEI-MS50 instrument.

2 - (3' - *Methyl)butanoyl* - 4 - (3' - *methyl)butyltetronic acid* (**5a** *and* **6a**)

1.81 g (5×10^{-3} mole) of **2a** were dissolved in 100 ml isopropanol together with 100 mg rose Bengal or methylene blue and 10% di-t-butylhydroxytoluene as triplet oxygen inhibitor. Oxygen is circulated through the solution, which is irradiated with a 1000 W Osram type 64741 lamp. The reaction course is followed by HPLC with the strong basic anion exchange resin Zerolit FFIP (particle size 8–12 μ) as stationary phase and a 90% methanolic acetate



5,6

R = -CH$_2$CH(CH$_3$)$_2$

R' = -CH$_2$CH$_2$CH(CH$_3$)$_2$ a, series

= -CH$_2$CH=C(CH$_3$)$_2$ b, series

Chart 1

buffer pH = 5.8 as eluting solvent. Separation is carried out by counter current distribution in the ether/phosphate buffer pH = 5.6 system after 550 transfers. The band with K = 0.1 is identified as the tautomeric mixture **5a** and **6a**. The yield is 8%. MS: *m/e* (%) 254 (1.1); 239 (0.5); 221 (0.5); 212 (0.5); 197 (0.6); 184 (4.8); 43 (100). The accurate mass of the parent ion is 254.15423, (calc. for $C_{14}H_{22}O_4$: 254.15180). NMR ($CDCl_3$): δ 0.93 (3H, d, J = 7 Hz); 1.04 (3H, d, J = 7 Hz); 1.2–1.4 (2H, m); 1.5–1.75 (2H, m); 1.85–2 (1H, m); 2.1–2.25 (1H, m); 2.76 (2H, d, J = 7 Hz); 4.48 (1/2 H, 2 × d, J = 4.5 and 8 Hz); 4.61 (1/2 H, 2 × d, J = 4.5 and 8 Hz); 12.57 (1H, s). UV: λ_{max} (ϵ) 265 (12,650) and 228 (10,200) nm in CH_3OH/0.1 N HCl; 262 (14,850) and 230 (12,375) nm in CH_3OH/0.1 N NaOH.

2 - (3' - *Methyl)butanoyl* - 4 - (3' - *methyl)but* - 2' - *enyltetronic acid* (**5b** *and* **6b**)

15 g (4.1×10^{-2} mole) of **2b** is boiled in 30 l buffer 0.2 M phosphate–0.1 M citrate of pH = 5.4 for 2.5 hr. The organic material is extracted with iso-octane after acidification to pH = 1. Counter current distribution in the two phase system iso-octane/citrate-phosphate buffer pH = 3.95 affords a single peak with K = 0.35, containing the tautomers **5b** and **6b**. The yield is about 0.5% based on the starting material. MS: *m/e* (%) 252 (10); 237 (0.4); 219 (6.3); 210 (1.2); 197 (2.4); 184 (52); 85 (22); 69 (100); 58 (21); 52 (8); 41 (42). The parent ion has an accurate mass of 252.13870 (Calc. for $C_{14}H_{20}O_4$: 252.13615). NMR (CCL_4): δ 1.01 (3H, d, J = 7 Hz); 1.03 (3H, d, J = 7 Hz); 1.64 (3H, d, J = 4 Hz); 1.70 (3H, d, J = 3 Hz); 2.17 (1H, m); 2.41–2.68 (2H, m); 2.66–2.85 (2H, m); 4.53 (1/2 H, 2 × d, J = 4.65 and 4.75 Hz); 4.65 (1/2 H, 2 × d, J = 5 Hz); 5.06 (1H, t, J = 7 Hz); 12.2 (1H, s). UV: λ_{max} (ϵ) 267.5 (9,700) nm in CH_3OH/0.1 N HCl and 260 (13,600) and 227.5 (11,500) nm in CH_3OH/0.1 N NaOH.

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