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ANTI-ISOHUMULONE AND ITS DERIVATIVES

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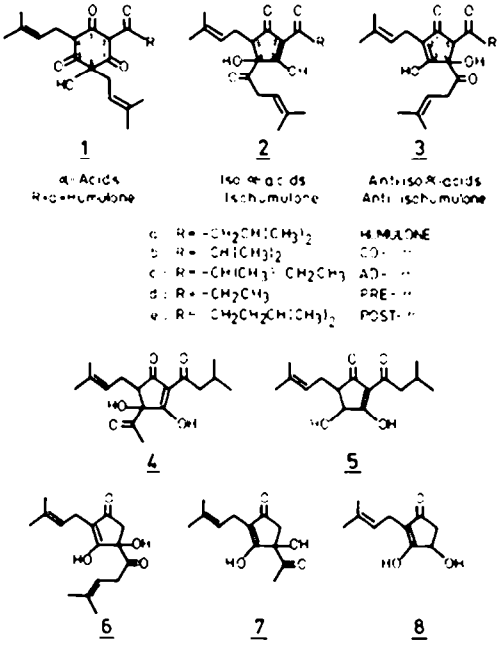
Abstract—Three new humulone derivatives have been isolated and identified as: 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 4 - (4 - methyl - 3 - pentenoyl) - 2 - cyclopentenone (**6**); 4 - ethanoyl - 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 2 - cyclopentenone (**7**) and 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 2 - cyclopentenone (**8**), respectively. They arise by deacylation of anti-isohumulone (**3a**), which is formed from humulone (**1a**) following an isomerization with ring contraction in opposite direction than the usual one producing isohumulones (**2a**).

The isomerization of the hop α-acids (**1**) occurs in the brewing process, thereby producing the iso-α-acids (**2**), which are the main bitter principles of beer. The reaction mechanism was shown to proceed via stereoselective ketonization of the enolate and subsequent acyloin ring contraction[2] to give the well known iso-α-acids (**2**) series. We now present an isomeric iso-α-acids series, which we will call the anti-iso-α-acids (**3**). These products can be isolated from α-acids isomerization mixtures obtained at various pH values. They are also formed in brewing circumstances and must therefore occur in beer. Humulone (**1a**), the major hop α-acid, contains in the ketonized form a double acyloin system. Ring contraction around the C_5–C_6 acyloin moiety affords the isohumulones (**2a**), while participation of the C_1–C_6 acyloin group would lead to the isomeric anti-isohumulones (**3a**). This structure has been invoked in the literature to explain the occurrence of two isohumulones,[3] but was rejected once their diastereoisomeric relationship was established.[4]



That the ring contraction occurs preferentially in one way is satisfactorily explained by the fact that the C_1-carbonyl group is part of a triacylmethane system. In isomerization conditions this is ionized and the negative charge prohibits approach of nucleophiles to this part of the molecule. The other acyloin moiety is therefore more reactive. The isomerization process in the brewery occurs however at pH of 5.2 i.e. below the pK_A value of the α-acids (e.g. **1a**: $pK_A = 5.5$), which must therefore partly prevail in the non-ionized form. This will also be the case at higher pH-values, although to a lesser extent. In this form maybe the C_1-carbonyl group can participate in an acyloin ring contraction, even at elevated pH-values. That this particular type of reaction happens indeed, has now been indirectly proven by isolating a series of hop bitter acids, which are derived from the still unknown anti-isohumulone (**3a**). The series consists of three new 5-membered ring compounds, analogous to the already known series of the isohumulones (**2a**),[4] the acetylhumulinic acids (**4**)[5] and the humulinic acids (**5**).[6] They are: 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 4 - (4 - methyl - 3 - pentenoyl) - 2 - cyclopentenone (**6**); 4 - ethanoyl - 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 2 - cyclopentenone (**7**) and 3,4 - dihydroxy - 2 - (3 - methyl - 2 - butenyl) - 2 - cyclopentenone (**8**), respectively. It should be noted that the same compounds are obtained from either homologue of the α-acids series.

These products are derived from **3a** by a deacylation reaction, which for this β-ketovinylogous acid or its conjugate base is in analogy with the well known easy fission of 1,3-dicarbonyl systems.[7] In the isohumulones (**2a**), this 3-methylbutanoyl side chain is not split off in similar conditions, because its carbonyl function is part of the triacylmethane system and bears a negative charge. Deacylation in the isohumulone series can occur after removing the acid hydrogen of the triacylmethane moiety. Typically this is best known in oxidation reactions occurring in strong alkaline oxidative medium,[8] in the Cu(II) ion catalyzed oxidation[9] and on heating in oxygen atmosphere.[10]

Compounds **6**, **7** and **8** are formed in highest yield when refluxing **1a** for 90 min in aqueous solution at pH = 11. They are isolated by ether extraction after thorough isooctane extraction of the acidified reaction mixture. The products are separated by counter current distribution in the two phase system ether: 0.25 M phosphate–citrate buffer with pH = 5.3 for the isolation of **6** (K = 0.96), pH = 4.1 for **7** (K = 0.48) and pH = 5.0 for **8** (K = 0.32), respectively. The new acids are crystalline and are fully characterized by elemental analysis and spectroscopic data.

The UV absorption maxima are located at 247.5–250 nm in acidic methanol and at 267 nm in alkaline methanol, in agreement with the calculated values for 5-membered

cyclic enones.[11] The mass spectra give the appropriate molecular ions and the most abundant ions arise from straightforward fragmentations. The ^{1}H NMR spectra provide explicit proof that the 3-methyl-butanoyl side-chain has been lost in the new series. A typical new feature is the AB-system, located between δ 2 and 3 and accounting for the ring methylene unit. The signals, due to the protons of the 3-methyl-2-butenyl group at C_2 and of the respective substituents at C_4 are readily attributed. The two possible enol tautomers of **6**, **7** and **8** interconvert very rapidly. Hence, an average signal for the methine proton attached to the carbon atom of the secondary alcohol function is seen in the ^{1}H NMR spectrum of **8**.

The acyloin ring contraction of **1a**, which gives compound **6** after deacylation of the presumably first formed anti-isohumulone (**3a**), occurs stereospecifically. The high optical rotation—$[\alpha]_D^{25°} = -125°$ for **6**—does not change on further recrystallization. The formation of **7** and **8** from **6** is completely analogous to the known isomerization and degradation sequence from **2a** to **4** and **5**.[4,5] Compound **6** is isomerized to the α-β-enone, which is subsequently hydrated. A retro-aldol reaction affords **7** and further deacetylation produces finally product **8**.

The combined yield of the new acids in the total reaction mixture is about 8.5%. Compound **8** is present for much less than 1%. This was not expected at all, since the analogous compound **4** affords very readily **5** and, as a consequence, can only be obtained in mild conditions.[5] The present case seems to be particular in that the acetyl derivative **7** is the most abundant member of the new series. In fact, quantitative transformation of **7** into **8** requires refluxing in 50% NaOH. This difference in behaviour between **7** and **4** may reflect the relative stability of the carbanion intermediately formed after deacylation. This carbanion of product **5** is in allylic position with respect to a triacylmethane enolate moiety, while it is next to a vinylogous carboxylate group in **8**.

The new compounds, derived from the hitherto unknown anti-isohumulone (**3a**), are about half as bitter as the isohumulones (**2a**).

EXPERIMENTAL

The UV spectra are recorded on a Cary 15 spectrophotometer, the 300 MHz ^{1}H NMR spectra on a Varian HA-300 machine (10% solution with TMS as internal reference) and the mass spectra on a AEI MS-50 mass spectrometer. The optical rotations are measured with a Perkin-Elmer Model 141 polarimeter. M.ps., determined with the help of a Kofler micro hot stage apparatus, are uncorrected. The preparative separations are carried out in a laboratory-built fully automatic CCD apparatus, containing 330 cells.

Preparation and isolation of **6**, **7** *and* **8**. The pH of an aqueous 2 l soln of about 0.055 mole potassium humulate (20 g of **1a**; 3.09 g KOH) was adjusted to 11 with 1N KOH. This clear soln was boiled for 90 min, subsequently cooled and acidified with ice-cold 2N HCl to pH = 1. The aqueous layer was thoroughly extracted with isooctane (4×) and then with freshly distilled ether (3×). The combined ether layers were dried over $MgSO_4$. Evaporation of the solvent left a brown cyrstalline residue (about 3 g), which was distributed in the two phase system ether: 0.25 M phosphate-citrate buffer. Compound **6** has a K-value of 0.96 at pH = 5.3 and was isolated after 2,000 transfers (0.343 g; yield: 2.2%). About 600 transfers at pH = 4.1 are needed to isolate product **7** with K = 0.48 (0.760 g; yield: 6.1%). Compound **8** has a K-value of 0.32 at pH = 5.0 (0.021 g; yield: 0.2%).

Identification of **6**. Compound **6** was recrystallized from CCl_4: $CHCl_3$ (m.p. 106°; $[\alpha]_D^{25°}$ in MeOH = −125°). The pK_A-value in MeOH : H_2O (1 : 1) is 4.06. (Found: C, 68.87; H, 7.71; Calc. for $C_{16}H_{22}O_4$: C, 69.04; H, 7.96%); UV: λ_{max} (ϵ): 250 (12,300) nm in 0.1 N HCl : MeOH and 267 (17,300) nm in 0.1 N NaOH : MeOH. ^{1}H NMR: δ ($CDCl_3$): 1.56 (3 H, s); 1.75 (3 H, s); 1.76 (6 H, s); 2.63; 2.85 (2 H, AB, J = −17.5 Hz); 2.96 (2 H, d, J = 7 Hz); 3.11; 3.24 (2 H, AB part of ABX, J_{AB} = −17.5 Hz; J_{AX} = 6.75 Hz; J_{BX} = 7.25 Hz); 5.21 (1 H, t, J = 7 Hz); 5.29 (1 H, X part of ABX, J_{AX} = 6.75 Hz; J_{BX} = 7.25 Hz). MS: *m/e* (%): 278(8); 260(3); 210(6); 209(5); 182(45); 181(21); 126(94); 69(96); 41(100).

Identification of **7**. Compound **7** was recrystallized from ether (m.p. 126°; $[\alpha]_D^{25°}$ in MeOH = −7.4°). The pK_A-value in MeOH : H_2O (1 : 1) was 4.08. (Found: C, 63.51; H, 7.01; Calc. for $C_{13}H_{18}O_5$: C, 64.27; H, 7.19%). UV: λ_{max} (ϵ): 250 (11,400) nm in 0.1 N HCl : MeOH and 267 (17,000) nm in 0.1 N NaOH : MeOH. ^{1}H NMR: δ ($(CD_3)_2CO$): 1.62 (3 H, s); 1.65 (3 H, s); 2.22 (3 H, s); 2.45; 3.00 (2 H, AB, J = −17.5 Hz); 2.83 (2 H, d, J = 7 Hz); 5.11 (1 H, s); 5.13 (1 H, t, J = 7 Hz). MS: *m/e* (%): 224(16); 209(1); 206(5); 191(3); 183(44); 182(14); 164(14); 155(6); 153(11); 126(54); 69(35); 43(100).

Identification of **8**. Compound **8** was recrystallized from ether (m.p. 104°). The pK_A-value in MeOH : H_2O (1 : 1) was 4.9. (Found: C, 65.49; H, 7.93; Calc. for $C_{16}H_{24}O_5$: C, 65.93; H, 7.69%). UV: λ_{max} (ϵ): 247.5 (15,600) nm in 0.1 N HCl : MeOH and 267.5 (25,000) nm in 0.1 NaOH : MeOH. ^{1}H NMR: δ ($CDCl_3$: $(CD_3)_2CO$, 20 : 1): 1.74 (3 H, s); 1.75 (3 H, S): 2.34; 2.78 (2 H, AB part of ABX, J_{AB} = −17.5 Hz; J_{AX} = 6.75 Hz; J_{BX} = 2.75 Hz); 2.84 (2 H, d, J = 7 Hz); 4.64 (1 H, X part of ABX, J_{AX} = 6.75 Hz; J_{BX} = 2.75 Hz); 5.16 (1 H, t, J = 7 Hz). MS: *m/e* (%): 182(84); 167(15); 164(14); 149(74); 121(34); 109(29); 81(26); 69(21); 56(47); 55(79); 43(100).

Preparation of **8** *from* **7**. 0.0364 g of **7** (2×10^{-4} mole) was dissolved in 5 ml 50% NaOH and the resulting soln boiled for 20 min. The cooled mixture was acidified with 2N HCl to pH = 1 and extracted several times with ether. The combined ether layers were dried over $MgSO_4$ and the solvent evaporated. The residue was recrystallized from a small amount of ether to give pure **8** (0.0275 g; yield: 95%).

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