Tetrahedron Vol. 35, pp. 989 to 992. Pergamon Press 1979. Printed in Great Britain

# ISOLATION AND IDENTIFICATION OF THE ANTI-ISOHUMULONES AND THE ANTI-ACETYLHUMULINIC ACIDS

LEO DE TAEYE, DENIS DE KEUKELEIRE[1] and MAURICE VERZELE*
State University of Ghent, Laboratory of Organic Chemistry, Krijgslaan, 271 (S.4), B-9000 Gent, Belgium

(*Received in UK 25 July 1978*)

Abstract—The anti-isohumulones [5-(3-methylbutanoyl)-2-(3-methylbut-2-enyl)-4-hydroxy-4-(4-methylpent-3-enoyl)-cyclopentane-1,3-diones] and the anti-acetylhumulinic acids [5-(3-methylbutanoyl)-2-(3-methylbut-2-enyl)-4-ethanoyl-4-hydroxy-cyclopentane 1,3-diones] have been isolated from an isomerisation reaction mixture of humulone [2-(3-methylbutanoyl)-4,6-di(3-methylbut-2-enyl)-6-hydroxy-cyclohexane-1,3,5-trione] by counter-current distribution and identified by spectroscopic techniques. The formation mechanism is presented and the stereochemical consequences are discussed. The anti-isohumulones are the most bitter hop compounds presently known.

During the brewing process the hop $\alpha$-acids (1, Fig. 1) are transformed into the iso-$\alpha$-acids (2, Fig. 1), which impart mainly the characteristic bitter taste to beer. The isomerisation yield of $\alpha$-acids can be as high as 85–90%, particularly in the presence of $Mg^{2+}$-ions,[2] but the effective utilisation in the brewery with hops is only 15–30%.[3] A beer, bittered with iso-$\alpha$-acids, does not taste exactly the same as a normally brewed beer.[4] Hence, bitter contributions must come from other sources. It has indeed been shown that compounds, occurring in beer, such as the allo-iso-$\alpha$-acids (3, Fig. 1),[5] deacylated anti-isohumulone (4, Fig. 1) and its derivatives[6] [deacylated anti-acetylhumulinic acid (5, Fig. 1) and decylated anti-humulinic acid (6, Fig. 1)], tricyclodehydroisohumulone (7, Fig. 1)[7] and the hulupones (8, Fig. 1),[8] have bittering properties. The bitter strength, however, is roughly only about half that of the iso-$\alpha$-acids, except maybe for the hulupones.

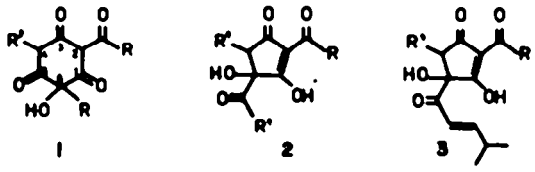
We now report the isolation and identification of the anti-isohumulones (9, 10, Fig. 2), which are twice as bitter as the iso-$\alpha$-acids and are therefore the most bitter hop acids actually known. The discovery of the anti-acetylhumulinic acids (11, 12, Fig. 2) is also described.

In a previous paper we have dealt with deacylated derivatives of the anti-isohumulones.[6] It was suggested that the anti-isohumulones themselves may also be present in the mixture, obtained by boiling humulone in aqueous buffer solution pH = 11. This is indeed the case. While the deacylated derivatives have been isolated from the ether extract,[6] the anti-isohumulones and the anti-acetylhumulinic acids are present in the iso-octane extract. The separation and isolation is achieved by counter-current distribution (CCD).

The compound with the smaller distribution coefficient is *cis* anti-isohumulone (9, Fig. 2), the other compound being the epimeric *trans* anti-isohumulone (10, Fig. 2). It follows from physical and spectral data (Experimental) that the chromophore is a 1,3-diketo moiety in a 5-membered ring.[9] The $^1H$ NMR spectra prove that the anti-isohumulones occur as a mixture of 2 enol tautomers, since the ring methine proton shows up as 2 singlets at $\delta$ 4.29 and $\delta$ 4.62 in a ratio 1:1 for 9 and at $\delta$ 4.72 and $\delta$ 5.32 in a ratio 3:2 for 10. The separate signals collapse upon addition of a trace of triethylamine or pyridine. The 6 Me groups absorb at different field, while the 3ABX-spin systems or subsystems, originating from protons in the side chains, are found at the appropriate positions in the spectra. Distinction between the *cis-trans* isomers, named according to the nomenclature adopted by the European Brewery Convention,[10] follows from IR and mass spectral data. In the *cis* isomer, an intramolecular hydrogen bridge is formed between the ring OH group and the CO group of the 3-methylbutanoyl side chain. Hence, in $5 \times 10^{-3}$ M $CCl_4$-solution, a broad OH-band persists between 3650 and 3100 $cm^{-1}$ in addition to a sharp signal at 3540 $cm^{-1}$, which is the only IR absorption band observed for the *trans* isomer. The configurational assignment from chemical ionisation mass spectral data is based upon the ratio of the relative abundances $(M+H)^+ : (M+H)^+ + (M-H)^+$. This ratio is a measure of the probability for formation of linear H-bonds,[11] which is, in this case, much more pronounced in the *cis* isomer 9. Hence, the $(M+H)^+$-ion is strongly stabilised, while such stereochemical feature does not exist for the $(M-H)^+$-ion. The experimentally found ratios m/z 363: m/z 363 + mz 361 are 0.89 for 9 and 0.39 for 10, which is quite relevant to discriminate between the isomers. Furthermore, the negative specific optical rotations at the Na–D line are consistent with the epimeric nature of the compounds.

The more stable *cis* anti-isohumulone (9, Fig. 2), carrying the 2 side chains at the chiral centres in *trans* position, outweighs the *trans* anti-isohumulone (10, Fig. 2) in the isomerisation mixture by a factor 20:1.

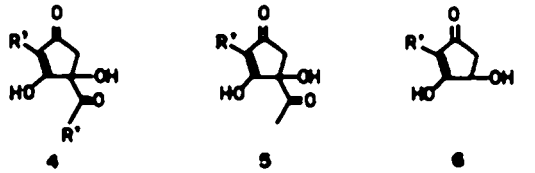
The weak basic isomerisation medium could yield also hydrolysis products of the anti-isohumulones, namely the anti-acetylhumulinic acids, as is found with the iso-humulones.[12] This again is indeed the case. Separation is carried out by CCD. The compound with the smaller distribution coefficient is *cis* anti-acetylhumulinic acid (11, Fig. 2), while the other band consists of *trans* anti-acetylhumulinic acid (12, Fig. 2). The physical and spectral data (Experimental) point again to a cyclopentane-1,3-dione basic structural unit.[9] Like the anti-isohumulones, each new compound occurs as a mixture of 2 enol tautomers, as is clearly illustrated in the $^1H$ NMR spectra. *trans* Anti-acetylhumulinic acid (12, Fig. 2) shows 2 singlets for the ring methine proton



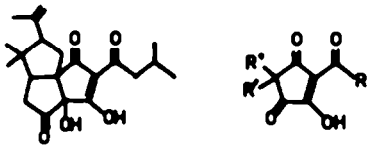
1 2 3

α-ACIDS ISO-α-ACIDS ALLO-ISO-α-ACIDS
R=a=HUMULONE ISOHUMULONE ALLO-ISOHUMULONE

a : R = -$CH_2CH(CH_3)_2$ : HUMULONE
b : R = -$CH(CH_3)_2$ : CO- "
c : R = -$CH(CH_3)CH_2CH_3$ : AD- "
d : R = -$CH_2CH_3$ : PRE- "
e : R = -$CH_2CH_2CH(CH_3)_2$ : POST- "



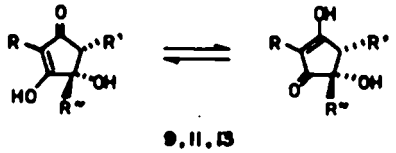
4 5 6



7 8

R' = -$CH_2CH=C(CH_3)_2$

Fig. 1.



9, 11, 13

10, 12, 14

R = -$CH_2CH=C(CH_3)_2$
R' = -$C(O)CH_2CH(CH_3)_2$
R'' = -$C(O)CH_2CH=C(CH_3)$ : 9, 10
-$C(O)CH_3$ : 11, 12
-H : 13, 14

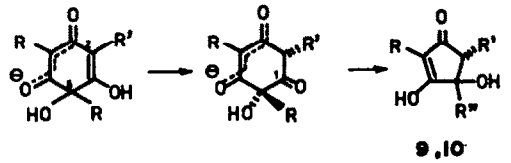
Fig. 2.

at $\delta$ 4.73 and $\delta$ 5.36 in a ratio of 6.5:3.5, while the corresponding signals for *cis* anti-acetylhumulinic acid (11, Fig. 2) are at $\delta$ 4.28 and $\delta$ 4.61 in a ratio of 1:1. The characteristic acetyl absorptions also occur as 2 separate signals in the same ratios: at $\delta$ 2.21 and $\delta$ 2.26 for 11 and $\delta$ 2.38 and $\delta$ 2.41 for 12. Small amounts of base cause collapse of the peaks. The appropriate signals are found for the 4 Me groups (2 doublets of doublets and 2 singlets) and for the ABX-spin systems or subsystems due to the methylene and methine protons of the side chains. The chemical ionisation mass spectral argument,[11] used to discern between the epimeric anti-isohumulones, holds here also. The ratio of the abundance of the $(M+H)^+$-ion, i.e. m/z 309, to the sum of the abundances of the $(M+H)^+$-and the $(M-H)^+$-ions, i.e. m/z 309 + m/z 307, is 0.9 for the *cis* isomer and 0.71 for the *trans* isomer. It is obvious that the *cis* compound is more apt to form a linear hydrogen bridge with a proton from the reactant gas than the corresponding *trans* derivative.

Interestingly enough, anti-humulinic acids (13, 14, Fig. 2) are not found in the reaction medium. Much stronger base is needed for the formation of these degradation products, as has been demonstrated in earlier work.[6]

The new compounds of the anti-series contain 2 chiral centres and occur as such in epimeric pairs. Apparently, enolisation within the 3-methylbutanoyl side chain, although feasible, does not occur. It is very likely that the "reversed" isomerisation of humulone follows the same reaction mechanism as the "normal" isomerisation, yielding the isohumulones, i.e. via stereospecific protonation at $C_2$ in humulone[13] (Fig. 3). The stereochemistry is governed by the requirement that the side chains at $C_2$ and $C_4$ be in *trans* position. Non-stereospecific ring contraction, involving the free CO group at $C_1$, leads to the epimeric mixture of the anti-isohumulones. The anti-acetylhumulinic acids are produced in a completely analogous way as shown for the formation of the acetylhumulinic acids, i.e. via double bond isomerisation and hydration in the 4-methylpent-3-enoyl side chain and retro-aldol reaction of the resulting $\beta$-hydroxyketones.[12]

The yields are low: 1.5% for the anti-isohumulones and 0.6% for the anti-acetylhumulinic acids. The reason for this is the enhanced difficulty of ketonising an enolised



9, 10

R=-$CH_2CH=C(CH_3)_2$

R'=-$C(O)CH_2CH(CH_3)_2$

R"=-$C(O)CH_2CH=C(CH_3)_2$

Fig. 3.

$\beta$-triketo system compared to an enolised $\beta$-diketo moiety. This phenomenon proceeds efficiently only under well defined oxidative conditions.[14] Consequently, the yield of the anti-isohumulones and derivatives is considerably lower than that of the isohumulones and derived products.

The importance of the anti-isohumulones lies in their extremely strong bittering power. Taste trials by a selected panel revealed that the anti-isohumulones are twice as bitter as the corresponding isohumulones, while the anti-acetylhumulinic acids have about half the bitterness of the isohumulones. The anti-isohumulones are now the most bitter hop acids known. Furthermore, the anti-isohumulones are more soluble in water than the isohumulones, which is demonstrated by the respective distribution coefficients in CCD. Hence, a better utilisation potential in the brewing process than the usual bitter acids is expected.

## 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), the mass spectra on a AEI MS-50 mass spectrometer and on a Finnigan 3200 chemical ionisation mass spectrometer, equipped with 6000 data system. The optical rotations are measured with a Perkin Elmer Model 141 polarimeter. The preparative separations were carried out in a laboratory-built fully automatic CCD apparatus, containing 400 cells.

*Preparation and isolation of* 9, 10, 11 and 12

The pH of an aqueous 2 l-solution of about 0.055 mole potassium humulate (20 g of 1a; 3.09 g KOH) was adjusted to 11 with 1 N KOH. This clear soln was boiled for 90 min, subsequently cooled and acidified with ice-cold 2 N HCl to pH = 1. The aqueous layer was thoroughly extracted with iso-octane (4x). The combined layers were dried over $MgSO_4$. Evaporation of the solvent left a brown residue, which was distributed in the two phase system ether: 0.25 M phosphate-citrate buffer. Compounds 9 and 10 have a K-value of 2.3 and 4, respectively, at pH 5.5 after 1850 transfers. Compounds 11 and 12 have a K-value of 0.75 and 1.16, respectively, at pH = 4.1 after 2300 transfers.

*Identification of* 9, yellow oil; $[\alpha]_D^{20}$ in MeOH = −56.3°; the $pK_A$-value in MeOH:$H_2O$ (1:1) is 3.3 (Found: C, 69.94; H, 8.76. Calc. for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34). UV: $\lambda_{max}$ ($\varepsilon$): 224 (11200) nm; 270 (9700) nm in 0,1 N HCl:MeOH and 255 (15900) nm, shoulder 270 nm in 0,1 N NaOH:MeOH. $^1$H NMR: $\delta$ ($CS_2$; pyridine; $CDCl_3$ 4:1:1): 0.90 (3H, d, J = 6.25 Hz); 0.91 (3H, d, J = 6.25 Hz); 1.40 (3H, s); 1.46 (3H, s); 1.52 (3H, s); 1.56 (3H, s); 2.13 (1H, m, X part of ABX system); 2.54–2.88 (4H, m, AB parts of 2 ABX systems); 3.13–3.29 (2H, AB part of ABX system; $J_{AB}$ = |18.5| Hz; $J_{AX}$ = 6.8 Hz; $J_{BX}$ = 7.2 Hz); 4.21 (1H, s); 4.98 (1H, X part of ABX system); 5.21 (1H, X part of ABX system); $\delta$ ($CDCl_3$); 4.29 (0.5 H, s); 4.62 (0.5 H, s). MS: m/z (%): 362 (11); 344 (2); 293 (12); 275 (25); 265 (60); 247 (30); 225 (14); 207 (24); 197 (18); 191 (45); 97 (4); 85 (50); 69 (100); 57 (45); 41 (79).

*Identification of* 10, yellow oil; $[\alpha]_D^{20}$ in MeOH = −7°. The $pK_A$-value in MeOH:$H_2O$ (1:1) is 3.15 (Found: C, 69.80; H, 8.94. Calc. for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34). UV: $\lambda_{max}$($\varepsilon$): 225 (10400) nm, shoulder 275 (5530) nm in 0, 1 N HCl:MeOH and 255 (14300) nm, shoulder 275 nm in 0,1 N NaOH/MeOH. $^1$H NMR: $\delta$ ($CDCl_3$) 0.99 (3H, d, J = 6 Hz); 1.01 (3H, d, J = 6 Hz); 1.6 (3H, s); 1.63 (3H, s); 1.65 (3H, s); 1.67 (3H, s); 2.77 (1H, m, X part of ABX system); 2.65–3.40 (4H, m, AB parts of 2 ABX systems); 3.1–3.5 (2H, m, AB part of ABX system); 4.72 (0.6 H, s); 4.97 (1H, m, X part of ABX system); 5.32 (1H, m, X part of ABX system); 5.34 (0.4 H, s). MS: m/z (%): 362 (8); 344 (2); 293 (16); 275 (18); 265 (33); 247 (33); 255 (24); 207 (29); 197 (6); 191 (14); 97 (15); 85 (18); 69 (13); 57 (49); 41 (100).

*Identification of* 11, yellow oil; $[\alpha]_D^{20}$ in MeOH = −18.8°; the $pK_A$-value in MeOH:$H_2O$ (1:1) is 3.8 (Found: C, 66.01; H, 7.63. Calc. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85). UV: $\lambda_{max}$ ($\varepsilon$): 225 (11580) nm, shoulder 265 (9220) nm in 0,1 N HCl:MeOH; 255 (14800) nm, shoulder 270 nm in 0,1 N NaOH:MeOH. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, 2xd); 0.99 (3H, 2xd); 1.69 (3H, s); 1.71 (3H, s); 2.17 (1H, m, X part of ABX system); 2.21 (1.5 H, s); 2.26 (1.5 H, s); 2.75–2.87 (4H, m, AB parts of 2 ABX systems); 4.28 (0.5 H, s); 4.61 (0.5 H, s); 5.03 (1H, 2xd, X part of ABX system). MS: m/z (%): 308 (1); 290 (2); 265 (57); 247 (60); 166 (23); 121 (33); 85 (21); 69 (34); 57 (36); 43 (100); 41 (23).

*Identification of* 12, yellow oil; $[\alpha]_D^{20}$ = −6.4° in MeOH; the $pK_A$-value in MeOH:$H_2O$ (1:1) is 3.7 (Found: C, 66.58; H, 8.03. Calc. for $C_{17}H_{24}O_5$: C, 66.21; H, 7.85). UV: $\lambda_{max}$ ($\varepsilon$): 227 (12400) nm, shoulder 256 (13060) nm in 0,1 N HCl:MeOH and 260 (14300) nm, shoulder 270 nm in 0,1 N NaOH:MeOH. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, 2xd); 0.99 (3H, 2xd); 1.58 (3H, s); 1.65 (3H, s); 2.17 (1H, m, X part of ABX system); 2.38 (1.05 H, s); 2.41 (1.95 H, s); 2.62–2.93 (4H, m, AB parts of 2 ABX systems); 4.73 (0.65 H, s); 4.96 (1H, 2xd, X part of ABX system); 5.36 (0.35 H, s). MS: m/z (%): 308 (3); 290 (2); 265 (55); 247 (47); 166 (27); 121 (24); 85 (56); 69 (98); 57 (60); 43 (100); 41 (95).

*Acknowledgements*—D. D. K. is indebted to the Belgian "National Fonds voor Wetenschappelijk Onderzoek" as a permanent research fellow of this foundation. The Belgian "Ministerie voor Wetenschapsbeleid" is thanked for financial help to the laboratory. We are grateful to Heineken Breweries–Rotterdam for supporting our hop research.

## REFERENCES

[1] *Bevoegdverklaard Navorser* of the Belgian *Nationaal Fonds voor Wetenschappelijk Onderzoek.*
[2] H. Köller, *Tetrahedron Letters* 4317 (1969).
[3] M. Verzele, *European Brewery Convention, Proceedings of the 10th Congress*, p. 400, *Stockholm*, 1965, Elsevier, Amsterdam (1966).
[4] M. Verzele, H. E. Jansen and A. Ferdinandus, *J. Inst. Brewing*, 76, 25 (1970).
[5] F. Alderweireldt, M. Verzele, M. Anteunis and J. Dierckens, *Bull. Soc. Chim. Belges* 74, 29 (1965); M. Verzele, M. Anteunis and F. Alderweireldt, *J. Inst. Brewing* 71, 232 (1965).
[6] L. De Taeye, D. De Keukeleire and M. Verzele, *Tetrahedron* 33, 573 (1977).
[7] J. A. Elvidge, D. R. Laws, J. D. McGuinness and P. V. R. Shannon, *Chem. & Ind.* 573 (1974); D. R. J. Laws and J. D. McGuinness, *J. Inst. Brewing* 80, 174 (1974); L. De Taeye, D. De Keukeleire, A. De Bruyn and M. Verzele, *Bull. Soc. Chim Belges* 87, 471 (1978).
[8] S. Brobult, R. Ryhage, L. O. Spetsig and E. Stenhagen, *European Brewery Convention, Proceedings of the 7th Congress*, p. 121, Rome, 1959, Elsevier, Amsterdam (1959).
[9] A. I. Scott, *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 58, Pergamon Press, Oxford (1964); E. Sieens, D. De Keukeleire and M. Verzele, *Bull. Soc. Chim. Belges* 85, 305 (1976).
[10] G. Krause, A. H. Cook and M. Verzele, *J. Inst. Brewing* 75, 340 (1969).
[11] A. Schwarz, U. Rapp and B. Windel, *Org. Mass Spectrosc.* 11, 219 (1976).

[12]M. Van Boven and M. Verzele, *Bull. Soc. Chim. Belges* 77, 99 (1968); *J. Inst. Brewing* 74, 81 (1968).
[13]M. Verzele and M. Van Boven, *Bull. Soc. Chim. Belges* 80, 677 (1971); M. Van Boven and M. Verzele, *Ibid.* 80, 197 (1971).
[14]G. Howard and J. Pollock, *J. Chem. Soc.* (C) 1902 (1952); E. Sinens, D. De Keukeleire and M. Verzele, *Tetrahedron* 33, 423 (1977).