The Anti-Iso-Alpha-Acids: Concept and Review of Current Research

Leo De Taeye, Denis De Keukeleire,[1] and Maurice Verzele,[2] *Laboratory of Organic Chemistry, State University of Ghent, Krijgslaan, 271 (S.4), B-9000 GENT (Belgium)*

ABSTRACT

Anti-iso-α-acids are positional isomers of iso-α-acids. They are formed in isomerization reaction mixtures of hop α-acids. The anti-isohumulones, 5-(3-methylbutanoyl)-2-(3-methyl-2-butenyl)-4-hydroxy-4-(4-methyl-3-pentenoyl)-cyclopentane-1,3-diones, and some of their derivatives were isolated from isomerization reaction mixtures of humulone. Total yield of the new compounds belonging to the antiseries exceeds 10%. Utilization of the antimembers in the brewing process is high. The anti-iso-α-acids are the most bitter-tasting hop acids known today.

Key words: *Anti-iso-α-acids, Deacylated anti-iso-α-acids, Hop α-acids, Iso-α-acids, Isomerization*

The importance of iso-α-acids (1, Fig. 1) in the beer brewing process is well established. Beer's bitter taste is mainly due to them (25), and other indispensable characteristics, such as the head retention of beer (4), are also related to these compounds. Minor contributors to beer bittering properties are: allo-isohumulones (2, Fig. 1) (1,2,24,25), hulupones (3, Fig. 1) (1,20,25), and some oxidation products of hop acids (17,27). Considerable efforts have been devoted to resolving the apparent discrepancy between the bitter taste of normally brewed beers and the taste obtained in beers brewed with the above mentioned compounds. As an example, tricyclodehydroisohumulone (4, Fig. 1), which recently became of some interest (7,13,16), shows interesting bitter characteristics.

In this paper, we describe a completely new and important series of hop acids discovered and studied in recent years at our laboratory. The parent compounds are the anti-isohumulones (5, Fig. 1), so-called because they arise from humulone (6, Fig. 1) via the inverse of the mechanism that forms isohumulones.

ANTI-ISOHUMULONES AND ANTI-ACETYLHUMULINIC ACIDS

Humulone (6, Fig. 2), the main hop α-acid, is always used as the substrate in our isomerization experiments. These are conducted in aqueous buffer solutions, either at pH 5.4 or 11. The exact conditions, resembling those prevailing in the brewing kettle, are described elsewhere (6,8,9,11). The acyloin isomerization of

compound 6 yields predominantly epimeric isohumulones via stereoselective ketonization at C-5 (23,26) (Fig. 2, path a). There is, a priori, no reason why the rearrangement could not take the inverse route, ie, via the carbonyl group at C-1 (Fig. 2, path b). For this reason, the two isohumulones were considered to be positional isomers at the time of their discovery (3,5). This postulation was dismissed after the isohumulones were proven to be epimers (2). Since then, the anti-isohumulones have been intensively searched for. The main difficulty was that the large excess of isohumulones prevented small amounts of isomers to be detected. Indeed, no doubt remains that ring contraction via C-5 is more likely, the C-1 keto function being part of a 1,3-tricarbonyl system. On the other hand, the use of model compounds (18) and deacylated analogues of hop bitter acids (12,15) has confirmed that an alternative process is feasible.

The low yield (1.5%) of the epimeric *cis* and *trans* anti-isohumulones (5, Fig. 2) (11) is accounted for by the difference in the ease of ketonizing a 1,3-dicarbonyl moiety vs a 1,3-tricarbonyl group. The latter phenomenon is, indeed, much more difficult to realize and has, up to now, only been observed in well-defined oxidative
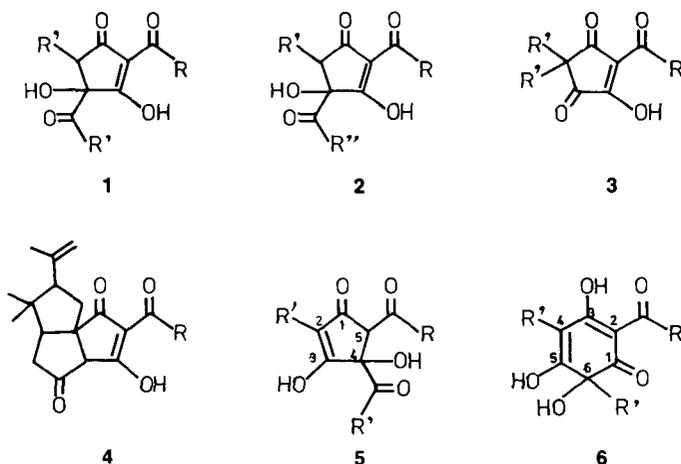
Fig. 1. Structures of some important hop acids derived from humulone (6). 1, Iso-α-acids; 2, allo-isohumulones; 3, hulupones; 4, tricyclodehydroisohumulone; 5, anti-isohumulones. R = $CH_2CH(CH_3)_2$, R′ = $CH_2CH=C(CH_3)_2$, R″ = $CH=CH-CH(CH_3)_2$.

circumstances (14,19). On the other hand, the better water-solubility of the anti-isohumulones implies that they have better potential for use in the brewing process than do the isohumulones (11). A highly reliable analysis of the anti-isohumulones in beer is, again, hampered by the overwhelming presence of the isohumulones, but we do have indications for their occurrence in beer.[3]

The great importance of the anti-isohumulones is their extremely pronounced bittering power. Results of taste trials by selected panels showed unequivocally that the *anti-isohumulones are twice as bitter as the isohumulones.* Consequently, the anti-iso-α-acids are the most bitter-tasting members of all the hop acids currently known. This useful property may be of considerable interest to the brewer, particularly if the ratio of the rearranged products could be altered in favor of the anti-isohumulones.

Experiments with this aim give a maximum ratio of anti-isohumulones to isohumulones of 2:3, using 50% aqueous dioxane as reaction medium and Mg(II)-salts as catalysts.[4] The anti-isohumulones (5, Fig. 3), which are formed directly from humulone (Fig. 3), are relatively unstable under the isomerization conditions and can react in several ways. First of all, the unsaturated acyl side chain at C-4 can show the same reaction sequence observed for the isohumulones (21,22), thus leading to the anti-acetylhumulinic acids (7, Fig. 3). The *cis* and *trans* epimers have been separated, purified, and completely characterized (yield: 0.2%) (11). Further degradation to the anti-humulinic acids (8, Fig. 3) occurs only in strong base (6).

DEACYLATED DERIVATIVES OF THE ANTI-ISOHUMULONES

Another degradation mechanism could start by deacylation of the side chain at C-5 in the anti-isohumulones. This reaction is, however, of minor importance and occurs with less than 2% yield (10). The result is a completely new series of deacylated derivatives, which we were able to isolate and identify (11). Deacylated anti-isohumulone (9, Fig. 3) (yield: 2.2%) is the parent compound. Modification and degradation of the 4-methyl-3-pentenoyl side chain at C-4 can lead to deacylated anti-acetylhumulinic acid (10, Fig. 3) in the same way as described previously (11,21,22). In contrast to the series of the isohumulones, compound 10 is the main representative (6.1%) of this series because further degradation to deacylated anti-humulinic acid (11, Fig. 3) is unlikely (11).

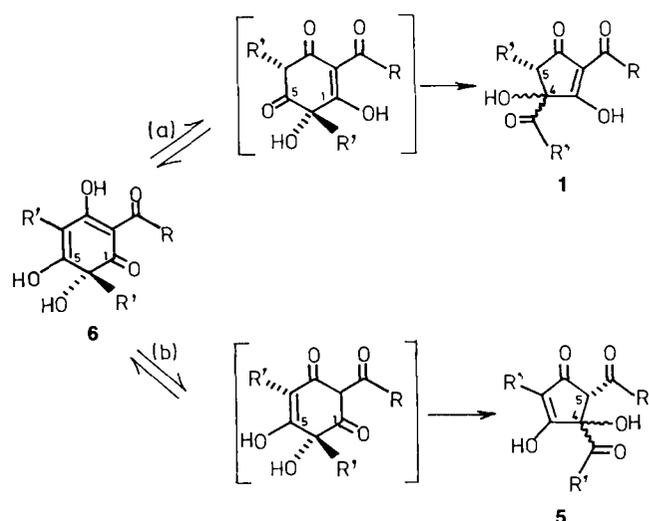[3] Unpublished results from this laboratory.
[4] Refer to note 3 above.

In fact, the yield of compound 11 is only 0.2%, although it can increase to nearly quantitative when compounds 9 or 10 are treated in strong alkaline medium. In the course of this reaction, racemization occurs.

The combined yield of compounds 9, 10, and 11 is about 8.5%, which is unexpectedly high in view of the fact that anti-isohumulones are relatively inert towards deacylation (10). As a consequence, the main formation pathway for this series must be via acyloin ring contraction of deacylated humulone (12, Fig. 3). Although this compound has not yet been found in isomerization mixtures of humulone, its intermediacy has been invoked or indirectly proven (12,15). Also, it is known to undergo isomerization, predominantly leading to the antiseries (10,12,15). The higher than expected overall yield of the deacylated antiderivatives is due to the tendency for humulone to be deacylated rather than to be isomerized by ring contraction via the carbonyl group at C-1.

The deacylated anti-derivatives do occur in beer, as would be expected from their polar character and fair solubility in water (6). The bitterness is about half that of the isohumulones; therefore the compounds contribute to the beer bitter taste to a certain extent.

Besides the two straightforward pathways shown in Fig. 3, anti-isohumulones are transposed into various classes of compounds, two of which have recently been characterized (9). When deacylated anti-isohumulone is transformed into deacylated anti-acetylhumulinic acid, it loses isobutyraldehyde, known in hop chemistry for its typical smell. Aldol-type condensation between these two degradation products yields compound 13. A complete new branch of compounds, derived from the anti-isohumulones, is represented by the heterocyclic furan derivative 14 (Fig. 3). The intricate formation mechanism is discussed in the literature (9). The
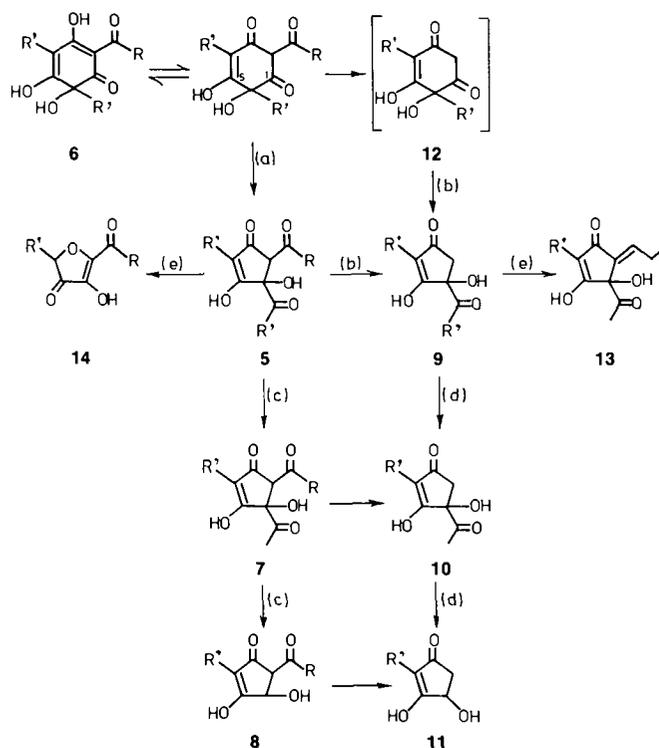
Fig. 3. Formation of the anti-isohumulones and derivatives. a, Formation of the anti-isohumulones (5) from humulone (6); b, pathway leading to deacylated anti-isohumulone (9) via deacylated humulone (12); c, degradation of the anti-isohumulones (5) to the anti-acetylhumulinic acids (7) and the anti-humulinic acids (8); d, degradation of deacylated anti-isohumulone (9) to deacylated anti-acetylhumulinic acid (10) and deacylated anti-humulinic acid (11); e, formation of the antiderivatives 13 and 14 from the anti-isohumulones (5) and deacylated anti-isohumulone (9), respectively. $R = CH_2CH(CH_3)_2$, $R' = CH_2CH=C(CH_3)_2$.

Fig. 2. Reaction pathways for the isomerization of humulone (6) into isohumulones (1) or anti-isohumulones (5). $R= CH_2CH(CH_3)_2$, $R' = CH_2CH=C(CH_3)_2$.

yield amounts to 0.3% each. Of course, these compounds are just two representatives of new classes belonging to the antiseries; other antiproducts may also be present in the isomerization reaction mixtures of humulone.

CONCLUSION

Anti-iso-α-acids and derivatives constitute an important fraction of the isomerization reaction mixture of hop-α-acids, not only because of the elevated total yield (>10%), but also because each individual member possesses pronounced bittering properties. The anti-iso-α-acids are the strongest bitter-tasting compounds known in hop chemistry. Some of the antiderivatives have been shown to occur in beer.

ACKNOWLEDGMENTS

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