Efficient One-Step Preparation of the Beer Additive Tetrahydroiso α-Acids

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α-Acids from hop extracts are simultaneously isomerized and hydrogenated to yield tetrahydroiso α-acids, an important additive in beer. The α-acids can also be sequentially isomerized and hydrogenated to tetrahydroiso α-acids, provided the hydrogenation is done between pH 6.0 and pH 11.0. The process is ideally suitable to scale-up and is more cost effective than any other route. The product has a higher purity than material produced by use of other reported methods. Hexahydroiso α-acids, a related beer additive, are also prepared in one step from α-acids.

Beer additives manufactured from isomerized and reduced hop extracts have gained major acceptance within the brewing industry in recent years (Verzele, 1979). One of the most commonly used additives is known as tetrahydroiso α-acids 4. This material is a significantly more potent bittering and foam-stabilizing agent than the nonmodified extracts. It also has increased photochemical and thermal stability due to the saturation of the side chains. Because of the growing demand for 4, we felt that it was important to develop a simpler preparation for this and related materials.

There are three reported routes to 4. The commercial synthesis (Worden and Todd, 1971; Worden, 1975; Cowles et al., 1986, 1987) uses the β-acid fraction (1) of the hop extract as the source material (Scheme I). This three-step process is inherently inefficient due to problems with the oxidation step, where overoxidation frequently occurs. Another synthesis involves an isomerization (Speitsig, 1964; Verzele and Van Boven, 1971; Verzele and Van Hoey, 1965; Alderweireldt et al., 1965; Carson, 1952) followed by a hydrogenation (Brown et al., 1959; Byrne and Shaw, 1971; Carson, 1952; Verzele and Govaert, 1947) of the α-acid fraction (5) of the hop extract. Isomerization of 5 is well-known and high yielding, resulting in commercial grade iso α-acids 6. The previous studies on the hydrogenation of 6, however, are confusing and sometimes contradictory. This may be because all natural hop acids contain at least three homologues, where the acyl side chain is a mixture of isobutyl, isopropyl, and sec-butyl (Scheme I). Consequently, the isolation and identification of products is difficult. The simple hydrogenation of 6 has never been reported in high yield, a fact that we have confirmed in our laboratory. A third synthesis of 4 involves a hydrogenation (Wollmer, 1916; Wieland et al., 1925; Carson, 1951; Riedl and Nickl, 1956; Verzele and Anteunis, 1959; Anteunis and Verzele, 1959; De Keukeleire and Verzele, 1970; De Keukeleire et al., 1976) of 5 followed by an isomerization (Worden, 1971). Verzele's papers on this route clearly indicate that side-chain cleavage leading to formation of quinone 8 is a major side reaction in the hydrogenation of 5. However, the amount of 8 produced could be minimized (Anteunis and Verzele, 1959) at pH >8. This hydrogenation requires very close monitoring of hydrogen uptake, as too much hydrogen results in overreduction and too little hydrogen results in incomplete reaction.
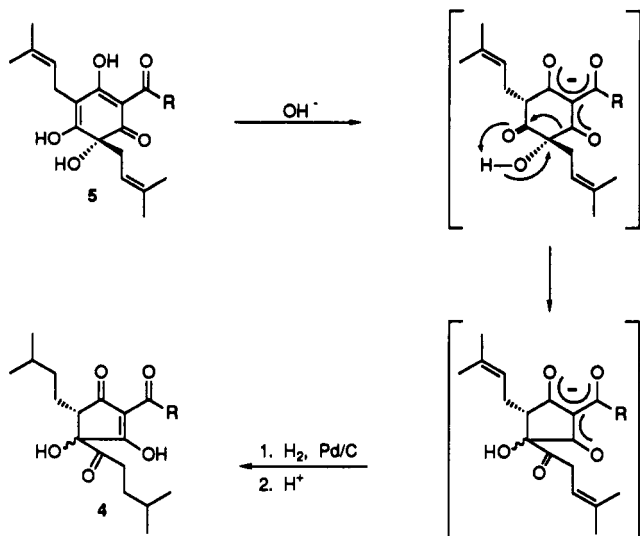
Scheme I

R = iBu, iPr, sec-Bu
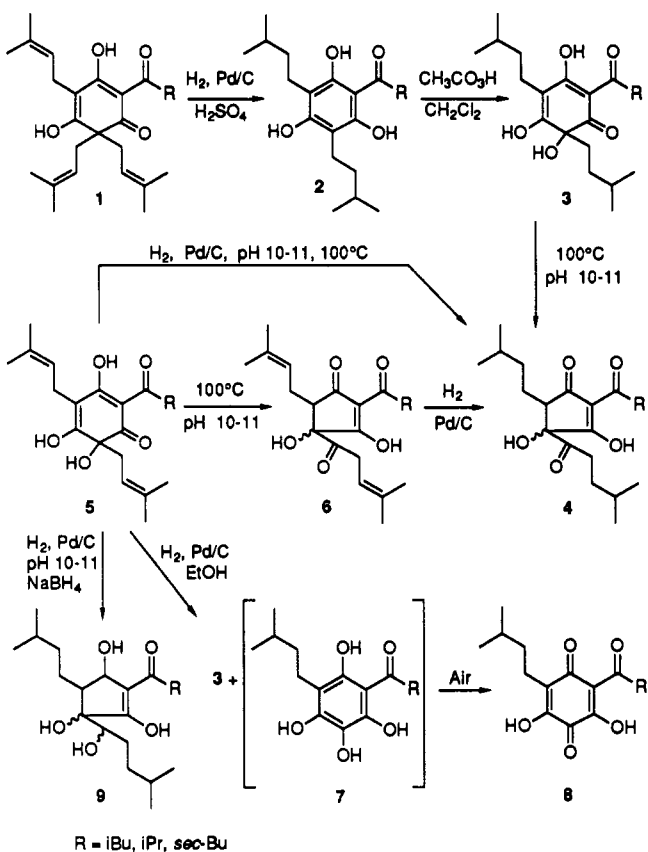
RESULTS AND DISCUSSION

We have found that the yield for the hydrogenation of 6 could be significantly improved by raising the pH of the reaction mixture above 5.5, the pK_a of 6. The pH of the oil in water before basification is typically 3.0 or less, because of impurities in the oil such as carboxylic acids and salts that are isolated along with 6 during the acidic precipitation. When the pH of the mixture was adjusted to between 6.0 and 11.0 with aqueous potassium hydroxide, the yield of the hydrogenation improved from 75% to 99%. This more facile route is economically superior to the route starting from 1 and is ideally suitable for scale-up.

A further advancement in this chemistry came with the discovery that 5 could be simultaneously isomerized and hydrogenated. While Verzele (Verzele and Anteunis, 1959; Anteunis and Verzele, 1959; De Keukeleire and Verzele,

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Scheme II

1970; De Keukeleire et al., 1976) reported that the hydrogenation of 5 in the synthesis of 3 was a capricious reaction yielding many side products, we have found that hydrogenation under isomerizing conditions (pH 10–11, 100 °C) resulted in high yields (92%) of 4. Presumably, the isomerization occurred much more rapidly than the hydrogenation, as under these conditions we have never observed any evidence for formation of quinone 8. The reaction time was not critical, and the pH could be easily maintained with a potassium carbonate buffer, making the reaction ideal for large-scale production.

This one-step synthesis of tetrahydroiso α-acids 4 is clearly the simplest and least expensive route known. The product synthesized by this improved method is of higher purity, as measured by HPLC, than 4 synthesized by any other reported route that does not involve chromatography or multiple recrystallizations. This is due to the efficiency of the synthesis. Because commercially prepared hop products are typically purified only by acid precipitation of the oils from an aqueous system, any impurities produced in the process, as well as other natural compounds in the original extract, may be contained in the final product. Other purification methods are impractical and are complicated by the fact that each product is a mixture of six components (cis and trans isomers of each of the three homologues). The one-step synthesis of 4 from α-acids 5 involves fewer steps than any other route, thus limiting the manipulations that can result in yield losses and lower purity levels. This higher purity product then has enhanced value in the brewing industry.

The new process material also contains a homologue ratio that mimics that of the starting α-acids. The β-acid 1 fraction typically contains more of the homologue in which R is isopropyl than the α-acids 5, which have the isobutyl R group fraction as the major component. Consequently, 4 produced from 1 has a homologue ratio much different from that of 4 produced from the α-acids 5, the source of the bittering agents in natural hops. The 4 synthesized from 5 also retains the chirality of the starting α-acids (Scheme II) (De Keukeleire and Verzele, 1971). Because an achiral intermediate is involved, all of the 4 produced from 1 is strictly racemic. Finally, the improved synthesis of 4 does not use any undesirable organic solvents, such as the methylene chloride employed in the literature β-acid-sourced process. This is significant, since it is important to keep toxic compounds out of food products.

A final extension of this technology was the discovery that hexahydroiso α-acids 9, a commercial product nor-

mally produced via a sodium borohydride reduction of 4 (Worden, 1971), could be synthesized in one step from 5 by using hydrogen, palladium on carbon, and sodium borohydride at pH 10–11 and 100 °C.

EXPERIMENTAL PROCEDURES

Yields were determined by HPLC peak area relative to analytically pure standards. HPLC analyses were run with an LDC/Milton Roy Constametric 3000 pump and Spectromonitor 3100 detector at 254 nm. A Waters Novapak C-18 column was used with a mobile phase of 60% 0.017 M (pH 7.4) tetrabutylammonium phosphate and 40% acetonitrile. Synthesis of pure standards of 4 will be described separately (Hay et al., 1991).

α-**Acids** 5 in aqueous solution were prepared from a liquid CO_2 extract of natural hops by using a pH-based aqueous separation (Cowles et al., 1986). HPLC quantification of 5 in solution was done vs a pure sample of a multiply recrystallized o-phenylenediamine salt of 5 (Alderton et al., 1954).

Iso α-acids 6 in aqueous solution were prepared from a solution of 5 via a base-catalyzed isomerization (Speitsig, 1964; Verzele and Van Boven, 1971; Verzele and Van Hoey, 1965). HPLC quantification of 6 in solution was done vs a pure sample of photoisomerized 5 (Clarke and Hildebrand, 1965).

Tetrahydroiso α-Acids 4 from Iso α-Acids 6. A 100-g aqueous solution at pH 10.0 containing 20.0% 6 by HPLC analysis was charged with 2.0 g of 10% palladium on carbon. The mixture was then hydrogenated on a Parr shaker at 50 psi and 100 °C for 6 h. The reaction was cooled, 100 mL of ethanol was added, and the catalyst was removed by filtration. The ethanol was removed by rotary evaporation, the solution was acidified to pH <2.0 with 50% sulfuric acid, and the oil was separated to yield 32.4 g of material assayed at 61% 4 by HPLC for a 99% yield from 6. Overnight vacuum desiccation of the oil gave a waxy product with a purity of 92% by HPLC.

Tetrahydroiso α-Acids 4 from α-Acids 5. A 100-g aqueous solution containing 14.4% 5 by HPLC analysis was adjusted to pH 10.5 with 20% aqueous potassium hydroxide and was then charged with 1.0 g of potassium carbonate and 1.0 g of magnesium chloride hexahydrate. After stirring, the pH was readjusted to 10.5 with 20% potassium hydroxide. The reaction was charged with 2.0 g of 10% palladium on carbon and was hydrogenated on a Parr shaker at 50 psi and 100 °C for 6 h. The reaction was cooled, 100 mL of ethanol was added, and the catalyst was removed by filtration. The ethanol was removed by rotary evaporation, the solution was acidified to pH <2.0 with 50% sulfuric acid, and the oil was separated to yield 20.5 g of material assayed at 65% 4 by HPLC for a 92% yield from 5. Overnight vacuum desiccation of the oil gave a waxy product with a purity of 93% by HPLC.

Hexahydroiso α-Acids 9 from α-Acids 5. A 100-g aqueous solution containing 16.4% 5 was charged with 50 mL of methanol, 1.0 g of potassium carbonate, and 1.0 g of magnesium chloride hexahydrate in 20 mL of water. The mixture was adjusted to pH 10.5 with 20% potassium hydroxide, and then 9.7 g of 12.0% sodium borohydride in 14 M sodium hydroxide was added, followed by 2.0 g 10% palladium on carbon. The mixture was placed on the Parr shaker with 10 psi of hydrogen and heated to 100 °C. As the pressure in the flask increased, it was vented to ensure that the pressure stayed below 50 psi. After 3 h, 2.0 g more of 10% palladium on carbon was added, and the hydrogenation was continued at 50 psi of hydrogen and 100 °C for 3 h. The mixture was cooled, 100 mL of methanol was added, and the catalyst was removed by filtration. The methanol was removed by rotary evaporation, the mixture was acidified to pH <2.0 with 50% sulfuric acid, and the oil was separated to yield 23.6 g of material. HPLC analysis indicated that 9 was the primary product.

ABBREVIATIONS USED

HPLC, high-pressure liquid chromatography; nm, nanometers; M, molar.

LITERATURE CITED

Alderton, G.; Bailey, G. F.; Lewis, J. C.; Stitt, F. Spectrophotometric Determination of Humulone Complex and Lupulone in Hops. *Anal. Chem.* **1954**, *26*, 983–992.

Alderweireldt, F.; Verzele, M.; Anteunis, M.; Dierckens, J. The Isomerization Products of Humulone. *Bull. Soc. Chim. Belg.* **1965**, *74*, 29–40.

Anteunis, M.; Verzele, M. On the Hydrogenation of Humulone. Part II. The Mechanism of Hydrogenolysis. *Bull. Soc. Chim. Belg.* **1959**, *68*, 476–483.

Brown, P. M.; Howard, G. A.; Tatchell, A. R. Chemistry of Hop Constituents. Part XIII. The Hydrogenation of isoHumulone. *J. Chem. Soc.* **1959**, 545–551.

Byrne, E.; Shaw, S. J. Reduction Products from *cis-* and *trans-*Isocohumulone. *J. Chem. Soc. C* **1971**, 2810–2813.

Carson, J. F. The Hydrogenation of Lupulone and Humulone. *J. Am. Chem. Soc.* **1951**, *73*, 1850–1851.

Carson, J. F. The Alkaline Isomerization of Humulone. *J. Am. Chem. Soc.* **1952**, *74*, 4615–4620.

Clarke, B.; Hildebrand, R. The Isomerization of Humulone I. Isolation of Photoisohumulone. *J. Inst. Brew.* **1965**, *71*, 26–36.

Cowles, J. M.; Goldstein, H.; Chicoye, E.; Ting, P. L. Process for Separation of Beta-Acids from Extract Containing Alpha-Acids and Beta-Acids. U.S. Patent 4,590,296, May 20, 1986.

Cowles, J. M.; Goldstein, H.; Chicoye, E.; Ting, P. L. Preparation of Tetrahydroisohumulones. U.S. Patent 4,644,084, Feb 17, 1987.

De Keukeleire, D.; Verzele, M. The Structure and Absolute Configuration of (–)-Humulone. *Tetrahedron* **1970**, *26*, 385–393.

De Keukeleire, D.; Verzele, M. The Absolute Configuration of the Isohumulones and the Humulinic Acids. *Tetrahedron* **1971**, *27*, 4939–4945.

De Keukeleire, D.; Siaens, E.; Verzele, M. Regiospecific Singlet Oxidation of (–) R-Tetrahydrohumulone and Hexahydrocolupulone. *Bull. Soc. Chim. Belg.* **1976**, *85*, 293–304.

Hay, B. A.; Homiski, J. W.; Priest, M. A. A Simplified Method for Synthesis of Tetrahydroisoalpha-Acid Standards for HPLC. *J. Am. Soc. Brew. Chem.* **1991**, in press.

Riedl, W.; Nickl, J. *Chem. Ber.* **1956**, *89*, 1838–1849.

Speitsig, L. O. Isolation and Characterization of Two Isohumulone Isomers. *J. Inst. Brew.* **1964**, *70*, 440–445.

Verzele, M. The Chemistry of Hops. In *Brewing Science*; Pollack, J. R. A., Ed.; Academic Press: New York, 1979; Vol. 1, pp 280–319.

Verzele, M.; Anteunis, M. On the Hydrogenation of Humulone. Part I. The Preparation of Tetrahydrohumulone. *Bull. Soc. Chim. Belg.* **1959**, *68*, 315–324.

Verzele, M.; Govaert, F. *Int. Congr. Ferment. Ind. Lect. Commun.* **1947**, 297–301.

Verzele, M.; Van Boven, M. The Isomerization of Humulone. *Bull Soc. Chim. Belg.* **1971**, *80*, 677–682.

Verzele, M.; Claus, H.; Van Hoey, M. Isomerization of the Alpha Acids of Hops. *Wallerstein Commun.* **1965**, *28*, 99–107.

Wieland, H.; Schneider, W.; Martz, E. *Chem. Ber.* **1925**, *58*, 102–112.

Wollmer, W. *Chem. Ber.* **1916**, *49*, 780–794.

Worden, L. R. Production of Hoplike Beverage Bittering Materials. U.S. Patent 3,923,897, Dec 2, 1975.

Worden, L. R.; Todd, P. H. Hop Flavors for Malt Beverages and the Like. U.S. Patent 3,522,975, Jan 5, 1971.