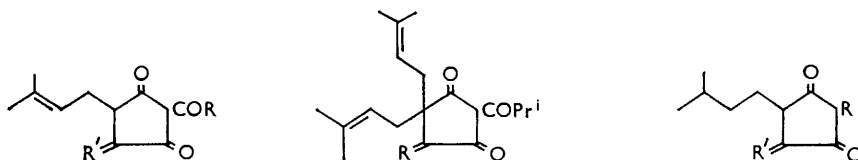


731. Chemistry of Hop Constituents. Part XX.¹ The Constitution of Humulinic Acid A and B as indicated by Proton Magnetic Resonance Spectroscopy.

By J. S. BURTON, J. A. ELVIDGE, and R. STEVENS.

Reduction of 3-isovaleryl-5-(3-methylbut-2-enyl)cyclopentane-1,2,4-trione with sodium borohydride affords humulinic acid B, isomeric with the known isomer, humulinic acid A, into which it is converted by means of basic reagents.² Similar reduction of isohumulonic acid affords dihydrohumulinic acid B. The proton resonance spectra of the different isomers are reported. The data show that the compounds have the *trans*- and *cis*-constitution, (XIII) and (XIV), respectively. The dihydrohumulinic acids A and B are the *trans*- and *cis*-isopentyl analogues, (XV) and (XVI). The enol tautomerism in the humulinic acids, and in the iso-, isoco-, and dehydro-humulinic acids receives comment.

THE most important bittering substances in beer are the poorly characterized, oily, isohumulones (I), produced by isomerization when the hop α -acids (humulone and its congeners) are boiled with wort. Similar isomerizations³⁻⁷ under mild alkaline conditions produce *inter alia* oils with similar chemical properties and bitterness; more drastic hydrolysis produces humulinic acid (II). Evidence from reversed-phase chromatography⁷ and counter-current distribution,⁸ however, suggests that each isohumulone is a mixture of two isomers. These have not, as yet, been separated on a preparative scale but, if this evidence is correct, hydrolysis of isohumulone should yield two isomers of humulinic acid. However, only one isomer of humulinic acid (II), m. p. 93°, which we now call humulinic acid A, was isolated in earlier studies.^{9,10} At the time this investigation started, Anteunis and Verzele^{11a} had presented evidence for the occurrence of a second isomer of humulinic



- (I: R = Bu^t, R' = HO, CO·CH₂·CH:CM₂)
 (II: R = Bu^t, R' = OH, H)
 (V: R = Bu^t, R' = O)
 (VI: R = Pr^t, R' = OH, H)
 (VII: R = Pr^t, R' = O)
- (III: R = O)
 (IV: R = OH, H)
- (VIII: R = CO·Bu^t, R' = OH, H)
 (IX: R = Bu^t, R' = OH, H)
 (X: R = CH₂Bu^t, R' = OH, H)
 (XI: R = CO·Bu^t, R' = O)
 (XII: R = CO·Pr^t, R' = O)

acid, humulinic acid B, m. p. 58—65°, in the mother-liquors obtained after treating humulinic acid A with alkali. In a subsequent Paper^{11b} they have described the isolation of pure humulinic acid B, m. p. 75°, by a lengthy counter-current distribution procedure.

We had shown earlier¹² that reduction of cohulupone (III) by sodium borohydride afforded the secondary alcohol (IV). Accordingly, reduction of the related triketone, 3-isovaleryl-5(3-methylbut-2-enyl)cyclopentane-1,2,4-trione (V) should lead to humulinic acid

¹ Part XIX, Stevens, J., 1964, 956.

² Preliminary communication: Burton and Stevens, *J. Inst. Brewing*, 1963, **69**, 200.

³ Windisch, Kolbach, and Schleicher, *Woch. Brau.*, 1927, **44**, 453 *et seq.*

⁴ Howard, *J. Inst. Brewing*, 1959, **65**, 414.

⁵ Carson, *J. Amer. Chem. Soc.*, 1952, **74**, 4615.

⁶ Howard, Slater, and Tatchell, *J. Inst. Brewing*, 1957, **63**, 237.

⁷ Spetsig, *Acta Chem. Scand.*, 1958, **12**, 592.

⁸ Whitear and Hudson, *J. Inst. Brewing*, 1964, **70**, 24.

⁹ Wieland and Martz, *Ber.*, 1926, **59**, 2352.

¹⁰ Spetsig, *J. Inst. Brewing*, 1963, **69**, 26.

¹¹ (a) Anteunis and Verzele, *Bull. Soc. chim. belges*, 1959, **68**, 102; (b) Anteunis, Bracke, Verzele, and Alderweireldt, *ibid.*, 1962, **71**, 623.

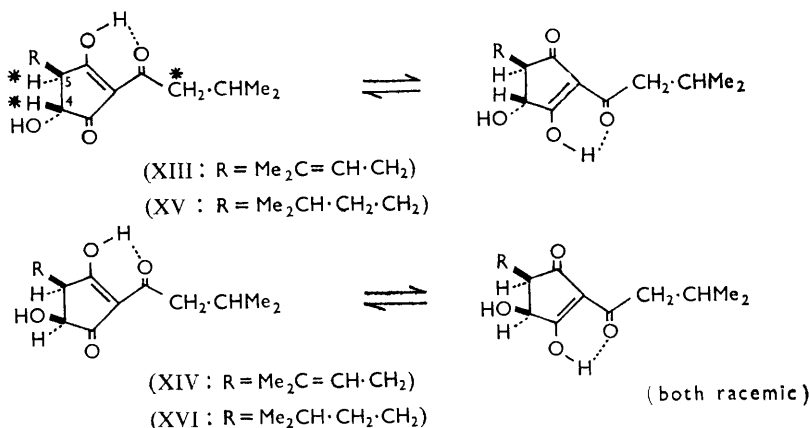
¹² Burton and Stevens, *J.*, 1963, 4382.

or its C₄-epimer. In fact we found that the product was entirely the isomeric acid, humulinic acid B, m. p. 73—4°. The ketone (V), for which we have elsewhere¹³ suggested the trivial name, dehydrohumulinic acid, is readily available by oxidation of humulinic acid A with bismuth oxide,¹⁴ so that our method is a much more convenient route to humulinic acid B than the counter-current distribution procedure.^{11b}

The ultraviolet absorption of humulinic acid B was identical to that of the A isomer; only small differences were seen in the infrared spectra of the two compounds, which were identical with those published subsequently.^{11b} Humulinic acid B was oxidized back to the triketone (V) by bismuth oxide, and treatment of humulinic acid B with sodium hydroxide or pyridine converted it into the A isomer; this explains why only traces of humulinic acid B are found among the hydrolysis products of humulone and isohumulone.

Similarly, cohumulinic acid A (VI) can be oxidized to dehydrocohumulinic acid (VII). This, when reduced with sodium borohydride, gave cohumulinic acid B, which was readily converted back into the A isomer with alkaline reagents.

Dihydrohumulinic acid A (VIII) has been obtained by hydrogenation of humulinic acid A, using colloidal palladium¹⁵ or Adams catalyst.^{16a} On one occasion hydrogenation of cohumulinic acid A (VI), with Adams catalyst, gave an abnormal product,^{16b} since shown to be (IX).¹⁷ Similar abnormal reductions have been found in the isohumulone¹⁷



and hulupone¹² series. In our hands the hydrogenation of humulinic acid A with 5% palladium-charcoal as catalyst, if stopped when 1 mole of hydrogen had been absorbed, affords dihydrohumulinic acid A. However, if the hydrogenation is allowed to go to completion the product is the abnormal reduction product (X) which we had encountered earlier when the triketone (V) was hydrogenated (Adams catalyst). Oxidation of dihydrohumulinic acid with bismuth oxide affords isohumulonic acid (XI).¹⁸

Reduction of isohumulonic acid (XI) by sodium borohydride gave two products: the abnormal reduction product (X) described above, and a second isomer of dihydrohumulinic acid—dihydrohumulinic acid B, m. p. 91.5—92°. This is most likely identical with the product, m. p. 88°, obtained by Harris, Howard, and Pollock^{16a} by hydrogenation of isohumulonic acid (Adams catalyst), but in our hands repetition of this hydrogenation gave only the abnormal product (X). Dihydrohumulinic acid B was also obtained by hydrogenation of humulinic acid B (5% palladium-barium sulphate). Treatment of dihydrohumulinic acid B with basic reagents affords the A isomer. Leucht and Riedl¹⁹ have

¹³ Forsén, Nilsson, Elvidge, Burton, and Stevens, *Acta Chem. Scand.*, 1964, **18**, 513.

¹⁴ Howard and Slater, *J.*, 1957, 1924.

¹⁵ Wöllmer, *Ber.*, 1916, **49**, 780.

¹⁶ (a) Harris, Howard, and Pollock, *J.*, 1952, 1906; (b) Howard and Tatchell, *J.*, 1954, 2400.

¹⁷ Brown, Howard, and Tatchell, *J.*, 1959, 545.

¹⁸ Howard, Pollock, and Tatchell, *J.*, 1955, 174.

¹⁹ Leucht and Riedl, *Annalen*, 1963, **669**, 55.

recently reported a synthesis of isohumulonic acid. According to our work, this also represents a synthesis of dihydrohumulonic acids A and B.²⁰

Dihydrocohumulonic acid A was readily obtained from colupulone by hydrogenation followed by hydrolysis.¹⁸ Oxidation of this dihydro-acid gave iso-cohumulonic acid (XII) which was reduced by sodium borohydride to dihydrocohumulonic acid B, m. p. 81—82°. During the preparation of dihydrocohumulonic acid A we encountered the product, m. p. 152—153°, described by Howard, Pollock, and Tatchell,¹⁸ and showed it to be the corresponding sodium salt; we also encountered the sodium salt of humulonic acid. Attempts to oxidize humulonic acid to the dehydrohumulonic acid with manganese dioxide, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), or nickel peroxide were unsuccessful.

That the humulonic and dihydrohumulonic acids A and B were C₄-epimers, as the chemical results suggested, was confirmed by proton magnetic resonance spectroscopy. This also showed that the carbonyl system is enolized differently from that in iso-, isoco-, and dehydro-humulonic acids.¹³

The proton signals from the side-chains of humulonic acid A (Table 1) are reminiscent of

Proton magnetic resonance results for 10% solutions in CDCl₃ containing 0.2% SiMe₄
 τ values (*J*, c./sec.).

Humulonic Acid		Dihydrohumulonic Acid		Intensity (splitting)	Assignment
A	B	A	B		
9.00(6.5)	9.01(6.5)	9.00(6.5)	9.01(6.5)	6 (doublet)	Me ₂ of isovaleryl chain
7.80 *	7.82	7.81	7.80	1 (multiplet)	CH of isovaleryl chain
7.20	7.22	7.22	7.20	2 (<i>ca.</i> doublet)	CH ₂ of isovaleryl chain
8.29	8.39, 8.33			6	Me ₂ of isopentenyl chain
4.83(7)	4.85(7)			1 (<i>ca.</i> triplet)	:CH of isopentenyl chain
7.47	7.58			2 (<i>ca.</i> triplet)	CH ₂ of isopentenyl chain
		9.04(5.5)	9.10(5.5)	6 (doublet)	Me ₂ of isopentyl chain
		8.33	8.33	} 5 (complex)	CH of isopentyl chain
		<i>ca.</i> 8.5	<i>ca.</i> 8.5		[CH ₂] ₂ of isopentyl chain
7.18	6.85	7.18	6.93 †	1 (double doublet)	5-H
5.83(4.5)	5.58(7.5)	5.95(4.5)	5.58(7.5)	1 (doublet)	4-H
0.68	0.80	0.83	0.92	2 (<i>ca.</i> 1.5 p.p.m. broad)	4-OH and enol (the positions were concentration-dependent)

* This origin position was confirmed by a decoupling experiment. Irradiation at a point 1.21 p.p.m. below the gem-dimethyl signal at τ 9.00 caused the latter to collapse to a singlet.

† The assignment was confirmed by irradiating at τ 6.93, whereupon the 4-H signal collapsed to a singlet.

those from the isovaleryl and isopentenyl chains of dehydrohumulonic acid (V),¹³ but some small differences are important. The signal from the methylene group in the 2-isovaleryl chain of humulonic acid A has a mean position 0.18 p.p.m. to lower field of the corresponding signal in dehydrohumulonic acid, in spite of the change at C₄. This indicates that the isovaleryl carbonyl is not enolized, in contrast to the situation in the dehydro-acid. We predict from Shooley's constants and the β -shift for a hydroxyl,²¹ that methylene protons adjacent to carbonyl would be less shielded than when adjacent to enolized carbonyl by about 0.28 p.p.m. The observed shift is in the correct direction but is smaller than predicted because there is no carbonyl group at C₄ in humulonic acid as there is at the corresponding 1-position in dehydrohumulonic acid. The signals from the 5-isopentenyl chain of humulonic acid are at higher field than those from dehydrohumulonic acid, particularly that from the methylene group, as appropriate for attachment to a saturated ring-carbon atom. This last methylene signal is split as a triplet (not as a doublet, as in dehydrohumulonic acid) because of approximately equal coupling to the adjacent olefinic proton and the proton at C₅. The signal from the latter is hidden beneath that from the methylene group of the isovaleryl chain, but its presence is revealed by the integral trace. From the

²⁰ Part XXI, Brown, Burton, and Stevens, *J.*, in the press.

²¹ Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, pp. 59, 53.

last and the expected width of the multiplet, the origin has been located. As expected, the proton at C₄ gives a sharp doublet (at τ 5.83).

At lower field there is a broad signal from the remaining two protons in humulinic acid A. One is the 4-hydroxyl proton and the other can only be that of the enolized 1,3-dicarbonyl system of the ring, and these must be exchanging.

The cyclopentenolone ring of humulinic acid A must be effectively planar, with the substituents at C₄ and C₅ eclipsed. The 4.5 c./sec. splitting of the 4-H signal points to a dihedral angle of 120° between 4- and 5-hydrogens,²² so that humulinic acid A has the *trans*-configuration (XIII).

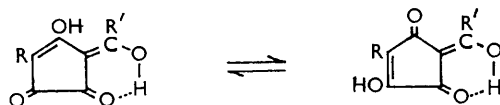
Humulinic acid B gives a similar spectrum except that the 4- and 5-proton signals are at lower field (so that the 5-H signal is unobscured) and *J* is 7.5 c./sec. This coupling points to a dihedral ^{4,5} angle of 0° between the 4- and 5-hydrogens²³ and so to a *cis*-configuration (XIV).

The relatively greater shielding of the 4- and 5-protons in the *trans*-isomer might be explained by the facts that the 4-proton can enter the shielding zone of the double bond in the 5-isopentenyl chain, and that the 5-proton is alongside the negative end of an O-C bond. However, different observations have been made in the cyclohexane series in respect of 1,3-substituents,²³ and it is uncertain at present whether the shielding effects are long-range in origin or derive from the geometry and anisotropy of the σ -framework.

The isomerization of humulinic acid B was performed also in alkaline deuterium oxide. The spectrum of the product (isolated by acidification with ordinary acid) resembled that of humulinic acid A with the signals from the four asterisked protons in (XIII) missing, these having been replaced by deuterium. The result showed that the alkali mobilizes the four protons adjacent to carbonyl groups, as expected, and as required at C₄ and C₅ for the *cis*- to *trans*-isomerization.

The spectra of the dihydrohumulinic acids (Table) showed that isomer A is the *trans*-compound (XV) and B the *cis* (XVI).

Finally, the difference between the enol signals from the humulinic acids and those from the iso-, isoco-, and dehydro-humulinic acids¹³ deserves comment. The single broad two-proton signal from the humulinic (and dihydrohumulinic) acids is consistent with these compounds undergoing 1,3-keto-enol tautomerism in solution because this would automatically involve the 4-hydroxyl proton in the exchange. The breadth of the signal means that the process does not fully average the protons' environments (because of rates, or residence times). The two enol signals from the other compounds indicate a lack of effective exchange between the protons concerned. This does not imply an absence of keto-enol



tautomerism; indeed this must occur because each signal is broadened. It is necessary to assume that the side-chain enol proton in iso-, isoco-, and dehydro-humulinic acids is tightly chelated (consistent with the strong deshielding) so that free exchange with the proton of the 1,3-keto-enol system of the ring is inhibited. The tautomerism (shown above) automatically gives two distinct environments for each enol proton, making the breadth of each signal explicable.

The spectra of *m*-hydroxybenzoic acid and 6-chloro-4-hydroxy-2-pyrone-3-carboxylic acid provide analogies. The former (in acetone) gives a single, broadened, two-proton signal near τ 1.0 (dependent upon concentration), indicative of exchange. The latter

²² Conroy in "Advances in Organic Chemistry," ed. Raphael, Taylor, and Wynberg, Interscience Publishers Inc., New York, 1960, 2, 265.

²³ Eliel, Gianni, Williams, and Stothers, *Tetrahedron Letters*, 1962, 741.

compound gives (in CDCl_3) two low-field signals at $\tau -4.25$ and -1.50 , attributable to the tightly, intramolecularly-chelated 4-hydroxyl and to the carboxyl proton, respectively.²⁴

EXPERIMENTAL

Light petroleum refers to the fraction b. p. $40-60^\circ$. Evaporations were carried out under nitrogen, and solutions were dried over anhydrous magnesium sulphate. For paper-chromatographic analysis Whatman's DE-20 paper was used with 25% acetic acid in methanol as solvent; R_F values refer to this system. Humulinic acid A,^{16a} m. p. $92-93^\circ$, R_F 0.14; dihydrohumulinic acid A,^{16a} m. p. $126-127^\circ$, R_F 0.15; cohumulnic acid A,^{16b} m. p. $79-80^\circ$, R_F 0.18; and dihydrocohumulnic acid A,¹⁸ m. p. $88-89^\circ$, R_F 0.22, were prepared as before. The ultraviolet absorption data for the individual humulinic acids are not given in detail as they were identical with those of humulinic acid A and B (cf. ref. 11b) within experimental error. Similarly the infrared spectra of humulinic acid A and B have been reported.^{11b}

On one occasion, during the preparation of humulinic acid A, the hydrolysed mixture was neutralized with insufficient acid and the sodium salt, m. p. $172-173^\circ$, was isolated (Found: C, 62.2; H, 7.6. $\text{C}_{15}\text{H}_{21}\text{NaO}_4$ requires C, 62.5; H, 7.3%).

Humulinic Acid B.—Dehydrohumulinic acid¹⁴ (0.74 g.) in ethanol (30 ml.), was added dropwise, with stirring, to sodium borohydride (0.55 g.) in water (10 ml.). Each addition was accompanied by a vigorous effervescence and the orange-red colour formed on the addition rapidly disappeared. The reaction mixture was stirred at room temperature for a further $2\frac{1}{2}$ hr., poured into water (100 ml.), and acidified with dilute hydrochloric acid. The precipitated oil was extracted into ether (3×30 ml.) and the combined ethereal extracts were washed with water, dried, and solvent removed to leave a solid residue. The solid was crystallized and recrystallized from cyclohexane to give *humulinic acid B* (0.23 g.), m. p. $72-73^\circ$, R_F 0.22 (Found: C, 67.4; H, 8.4. $\text{C}_{15}\text{H}_{22}\text{O}_4$ requires C, 67.6; H, 8.3%).

Humulinic acid B (0.07 g.) in 2N-sodium hydroxide (5 ml.) was heated under reflux for 1 hr. The reaction mixture was acidified and extracted with ether (3×15 ml.). The combined ethereal extracts were washed with water (10 ml.), dried, and evaporated to leave a solid residue. On recrystallization from cyclohexane, humulinic acid A (0.04 g.), m. p. and mixed m. p. $91-92^\circ$, was obtained.

Humulinic acid B (0.111 g.) in pyridine (5 ml.) was allowed to stand at room temperature for 3 days. The mixture was poured into water, acidified with dilute hydrochloric acid, and the precipitated oil extracted into ether (3×15 ml.). The combined ethereal extracts were washed with water (10 ml.), dried, and the solvent evaporated to leave crystals of humulinic acid A (0.05 g.), which on recrystallization from cyclohexane had m. p. and mixed m. p. $91-92^\circ$.

Humulinic acid B (0.04 g.) and bismuth oxide (0.08 g.) were heated together under reflux in glacial acetic acid (5 ml.) for 5 hr., poured on to ice-cold dilute hydrochloric acid (25 ml.), and extracted with ether (3×15 ml.). The combined ethereal extracts were washed with water (2×10 ml.), dried, and evaporated to leave yellow crystals of dehydrohumulinic acid (V) which on recrystallization from light petroleum (0.020 g.) had m. p. and mixed m. p. $133-134^\circ$.

Hydrogenation of Dehydrohumulinic Acid (V).—Dehydrohumulinic acid (0.33 g.) in methanol (30 ml.) was shaken in an atmosphere of hydrogen in the presence of Adams catalyst, until uptake was complete. After removal of the catalyst and solvent, the solid residue was recrystallized from ether-light petroleum and aqueous methanol to give 4-hydroxy-2,5-di-isopentyl-cyclopentane-1,3-dione (X) (0.11 g.) as colourless needles, m. p. $171-172^\circ$ (Found: C, 71.0; H, 10.2. $\text{C}_{15}\text{H}_{26}\text{O}_3$ requires C, 70.8; H, 10.25%). Light absorption: λ_{max} , 250 m μ (ϵ 15,700) in acidified ethanol, λ_{max} , 273 m μ (ϵ 23,500) in alkaline ethanol.

Hydrogenation of Humulinic Acid A.—(i) Humulinic acid A (0.214 g.) in methanol (20 ml.) was shaken in an atmosphere of hydrogen, in the presence of Adams catalyst until uptake of hydrogen ceased. The catalyst and solvent were removed to leave a solid residue. Recrystallization from ether-light petroleum gave dihydrohumulinic acid A (0.06 g.), m. p. and mixed m. p. $123-124^\circ$.

(ii) Humulinic acid A (3.0 g.) in methanol (100 ml.) was shaken with hydrogen in the presence of 5% palladium-charcoal. Hydrogen (348 ml.) (calc. for 1.0 double bond: 294 ml.) was taken up within 16 min. and the hydrogenation was stopped. After removal of catalyst the filtrate was concentrated and dihydrohumulinic acid, m. p. $125-127^\circ$, crystallized out (2.10 g.).

²⁴ Butt, Elvidge, and Foster, *J.*, 1963, 3069.

(iii) Humulinic acid A (503 mg.) in methanol (10 ml.) was hydrogenated in the presence of 5% palladium-charcoal (54 mg.) until hydrogen uptake ceased (169 ml. in 5 hr. Calc. for 3·0 double bond: 146 ml.). After removal of catalyst and solvent the residue (447 mg.) was recrystallized from ether-light petroleum and aqueous methanol to give 4-hydroxy-2,5-di-isopentylcyclopentane-1,3-dione, having m. p. and mixed m. p. 162°, identical with the sample obtained above.

Hydrogenation of Isohumulinic Acid.—The acid (0·16 g.) in methanol was shaken in an atmosphere of hydrogen in the presence of Adams catalyst. When hydrogen uptake ceased, catalyst and solvent were removed and the residue recrystallized from cyclohexane and aqueous methanol to give 4-hydroxy-2,5-di-isopentylcyclopentane-1,3-dione, m. p. and mixed m. p. 171·5—172·5°.

Hydrogenation of Dihydrohumulinic Acid A.—Dihydrohumulinic acid A (2·0 g.) in methanol (180 ml.) was shaken in an atmosphere of hydrogen in the presence of Adams catalyst, until uptake of hydrogen was complete. After removal of the catalyst and solvent, recrystallization of the residue from ether-light petroleum and aqueous methanol gave 4-hydroxy-2,5-di-isopentylcyclopentane-1,3-dione (X) (0·54 g.), m. p. and mixed m. p. with material obtained above 169—170°.

Reduction of Isohumulinic Acid.—A solution of isohumulinic acid ^{11a} (500 mg.) in ethanol (35 ml.) was added in small portions to a solution of sodium borohydride (355 mg.) in water (10 ml.) with stirring. After 2·5 hr. the mixture was decomposed with glacial acetic acid, diluted with water (300 ml.), and extracted with ether. Evaporation of the dried ethereal extracts gave a solid residue, part of which dissolved in cyclohexane (100 ml.). The residue after recrystallization from ether-light petroleum gave 5-hydroxy-2,4-di-isopentylcyclopentane-1,3-dione (103 mg.), m. p. and mixed m. p. 173—174°, having light absorption identical with the sample obtained as above. Evaporation of the cyclohexane solution gave *dihydrohumulinic acid B* (209 mg.) which after recrystallization from aqueous methanol had m. p. 91·5—92°, R_F 0·21 (Found: C, 67·5; H, 9·2. $C_{15}H_{24}O_4$ requires C, 67·2; H, 8·95%).

Dihydrohumulinic acid B (100 mg.) was heated under reflux with 2N-sodium hydroxide (5 ml.), and the solution was diluted with water and acidified. Extraction with ether and evaporation of the dried extract afforded dihydrohumulinic acid A (81 mg.) which after recrystallization from aqueous methanol had m. p. 124—125°.

Hydrogenation of Humulinic Acid B.—Humulinic acid B (0·1 g.) in methanol (20 ml.) was shaken in an atmosphere of hydrogen in the presence of 5% palladium-barium sulphate (50 mg.) until uptake ceased. The catalyst and solvent were removed, and the resulting solid was recrystallized twice to give dihydrohumulinic acid B, m. p. 98—100° (undepressed on admixture with material m. p. 92° obtained above. The two materials had the same R_F when examined by paper chromatography) (Found: C, 66·5; H, 9·0%).

Cohumulinic Acid B.—Dehydrocohumulinic acid ²⁰ (0·57 g.) in methanol (20 ml.) was added dropwise with stirring at room temperature to sodium borohydride (0·57 g.) in water (20 ml.). Each addition was accompanied by a vigorous effervescence, and the orange colour formed on each addition rapidly disappeared. The reaction mixture was stirred at room temperature for 4 hr. and then poured into ice-cold dilute hydrochloric acid (50 ml.). The precipitated oil was extracted into ether (3 × 30 ml.) and the combined ethereal phases were washed with water, dried, and the solvent removed to leave a red syrup. The syrup was distilled, b. p. 130—135° (bath)/5 × 10⁻⁴ mm. to give a pale yellow oil which deposited colourless crystals. Recrystallization from cyclohexane afforded *cohumulinic acid B* (0·04 g.) m. p. 67—68°, R_F 0·24 (Found: C, 66·3; H, 7·95. $C_{14}H_{20}O_4$ requires C, 66·7; H, 7·95%).

Cohumulinic acid B (0·02 g.) in 2N-sodium hydroxide (30 ml.) was heated under reflux for 1 hr. The reaction mixture was acidified and extracted with ether (3 × 15 ml.). The combined ethereal extracts were washed with water, dried, and evaporated to leave an orange syrup. From a solution in cyclohexane (0·5 ml.), crystals of cohumulinic acid A (10 mg.) were deposited, m. p. and mixed m. p. 78—79°.

Isocohumulinic Acid.—Dihydrocohumulinic acid A, ¹⁸ (2·93 g.) in glacial acetic acid (100 ml.) was heated under reflux for 19 hr. with bismuth oxide (3 g.). The reaction product was added to a mixture of ice and 2N-hydrochloric acid (100 ml.) and then extracted with ether (3 times). The ethereal extracts were washed with water (3 times), dried, and evaporated to afford isocohumulinic acid (2·72 g.) which after two recrystallizations from light petroleum had m. p. 120° (Found: C, 66·4; H, 8·0. Calc. for $C_{14}H_{20}O_4$: C, 66·6; H, 8·0%).

Dihydrocohumulinic Acid B.—A solution of sodium borohydride (350 mg.) in water (20 ml.)

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was added dropwise to a stirred solution of isocohumulonic acid (500 mg.) in ethanol (25 ml.). After 2 hr., excess of reagent was decomposed with 2*N*-hydrochloric acid, and the mixture diluted with water and extracted with ether. The dried ethereal extracts were evaporated and the residue extracted (4 times) with boiling light petroleum. The petroleum-soluble fraction (194 mg.) partially solidified and was distilled (b. p. 130°/10⁻² mm.). After storage, the solid material was separated and recrystallized from methanol-water to afford *dihydrocohumulonic acid B*, m. p. 81—82°, *R_F* 0.28 (Found: C, 66.1; H, 8.9. C₁₄H₂₂O₄ requires C, 66.1; H, 8.65%).

Dihydrocohumulonic acid B (15.5 mg.) in 2*N*-sodium hydroxide (2 ml.) was heated under reflux for 1 hr. The mixture was acidified with dilute hydrochloric acid and extracted with ether (3 × 10 ml.). The combined ethereal extracts were washed with water (10 ml.), dried, and evaporated to leave a semi-solid oil. Crystallization from aqueous methanol gave dihydrocohumulonic acid A (2 mg.) which had the same *R_F* as an authentic specimen when examined by paper chromatography.

Treatment of Humulonic Acid B with Sodium Deuterioxide.—A solution of sodium metal (0.46 g.) in deuterium oxide (10 ml.) was added to humulonic acid B (0.182 g.) and the mixture was heated under reflux for 1 hr. Dilute sulphuric acid was added until the solution was just acidic and the precipitated oil was extracted into ether (3 × 15 ml.). The combined ethereal phases were dried and evaporated. The solid residue was recrystallized from cyclohexane to leave the deuterated humulonic acid A (XIII, *H = D) (0.034 g.), m. p. 86—88°, which had τ (in CDCl₃) 9.00 (doublet, *J* 6.5 c./sec.), 7.822 (multiplet) (Me₂ and CH, respectively, of isovaleryl chain); 8.30, 7.45 (multiplet), 4.85 (*ca.* triplet) (Me₂, CH₂, and CH, respectively, of isopentyl chain); 1.33 (broad, concentration-dependent; 2 OH's).

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[Note added in proof (August 24th, 1964): Similar conclusions regarding the structures of humulonic acid A and B have been reached by Lepoivre, Alderweireldt, Anteunis, and Verzele (*Bull. Soc. chim. belges*, 1964, **70**, 275) and Alderweireldt and Anteunis (*ibid.*, 1964, **70**, 285.)]

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