

The Structure of Isohumulinone A, a Product derived by Oxidation and Isomerisation of Humulone from Hop Resin

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Spectroscopic evidence leads to a radical revision of the accepted structure of isohumulone A. This is supported by the properties of a perhydro-derivative, formed by hydrogenation over platinum oxide, and by mechanistic considerations. A new structure for isohumulone B is also suggested.

It is well known that the humulone content of hops falls during storage but that there is usually no corresponding fall in the bittering power of the hops. Amongst the many bitter oxidation products of humulone, the racemic humulinone (1) was described¹ in 1960 and the stereochemistry of this compound, as shown, was established by 1965.² Two further transformation products, isohumulones A and B of which B is bitter, were subsequently recognised,³ formed by treatment of humulinone (1) with acid. We have repeated the preparation of isohumulones A and B, using formic acid in boiling benzene, and have confirmed that these products are isomeric, each having the molecular formula $C_{21}H_{30}O_6$, but we disagree with the structures (2) and (3) previously proposed³ on limited evidence.

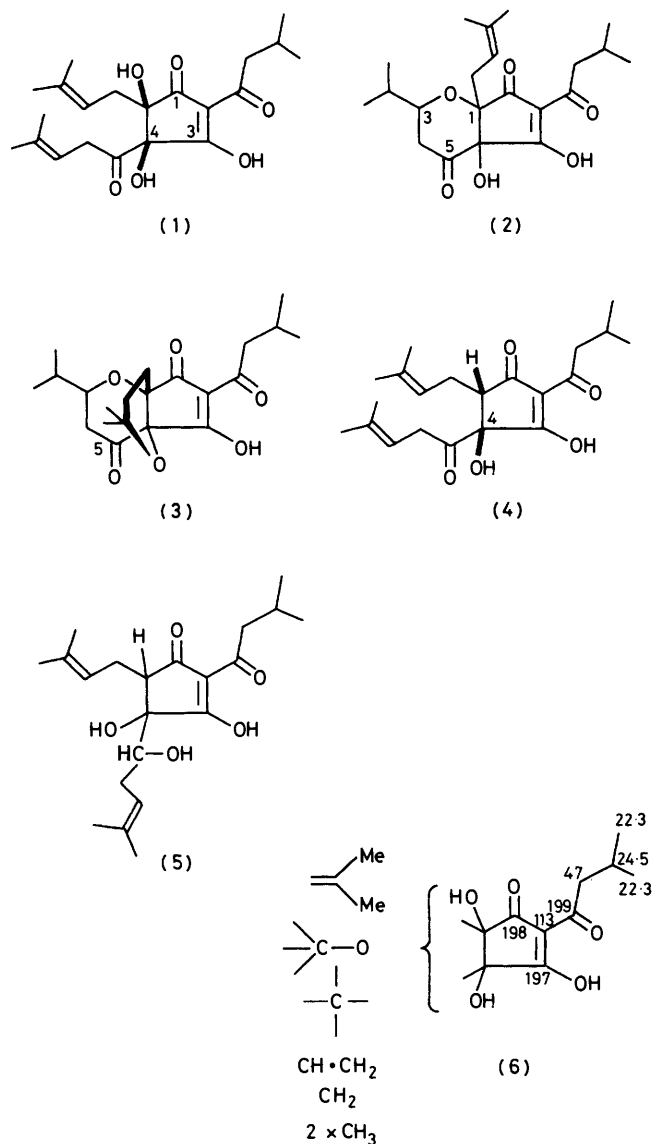
An immediate conflict with structure (2) was the failure of isohumulone A to undergo reduction at the 5-carbonyl group with sodium borohydride, strongly in contrast to photoisohumulone (4) and humulinone (1) itself. Thus photoisohumulone (4) was reduced readily (1.5 h) and the product (5) showed in its 1H n.m.r. spectrum the complete removal of the methylene signal of the 4-isohexenoyl group [δ 3.30 in (4)] and its replacement by a multiplet (1 H) at δ 3.78, due to the new α -carbonyl H atom of (5). The retention of the carbonyl of the 2-methylbutanoyl group in (5) was evident from its characteristic methylene doublet signal at δ 2.77. The mass spectrum of the product (5) showed M^+ 364 and the loss of 98 a.m.u. as a result of fragmentation of the new 1-hydroxy-4-methylpent-3-enyl side-chain with hydrogen rearrangement (*cf.* ref. 4). Reduction of humulinone (1) under identical conditions proceeded similarly but more slowly (4 h), whereas isohumulone A was recovered unchanged (1H n.m.r. and mixed m.p.) from the reaction mixture after 12 h. Moreover, the 1H n.m.r. spectrum (at 90 MHz) of isohumulone A was inconsistent with structure (2). Although the spectrum showed the characteristic signals from the isopropyl and methylene groups in the 3-methylbutanoyl side-chain, and two methyl signals attributable to an isopropylidene group, there was no vinylic proton signal, and the remaining two methyl signals were evidently derived from slightly non-equivalent methyl groups attached to a quaternary carbon atom. The broad low-field signal integrated for three enolic protons. These facts necessitated a revision of the structure (2).

The u.v. spectrum of isohumulone A closely resembled that of humulinone (1) suggesting that the 5-membered β -tricarboxyl ring system had been retained. In the noise decoupled ^{13}C n.m.r. spectrum, of the 21 signals only three were in the C=O region and three in the C=C region (δ 110–150). The loss of one double bond and of the C=O group of the isohexenoyl substituent of humulinone (1) implied *two* further rings in the structure of isohumulone A and hence tricyclic structures were considered. In the ^{13}C off-resonance spectrum (see Table) the two carbon signals of the C=C double bond at δ 123.8 and 144.9 remained as singlets, confirming that this double bond was tetrasubstituted. Similarly, three signals at δ 82.9, 86.6, and 87.4, also unsplit in the off-resonance mode, revealed the presence of three tetrasubstituted carbons linked to oxygen. These features greatly reduced the number of structural possibilities. The full spectral analysis is given in the Table.

^{13}C N.m.r. results for isohumulone A (7) in $(CD_3)_2SO$

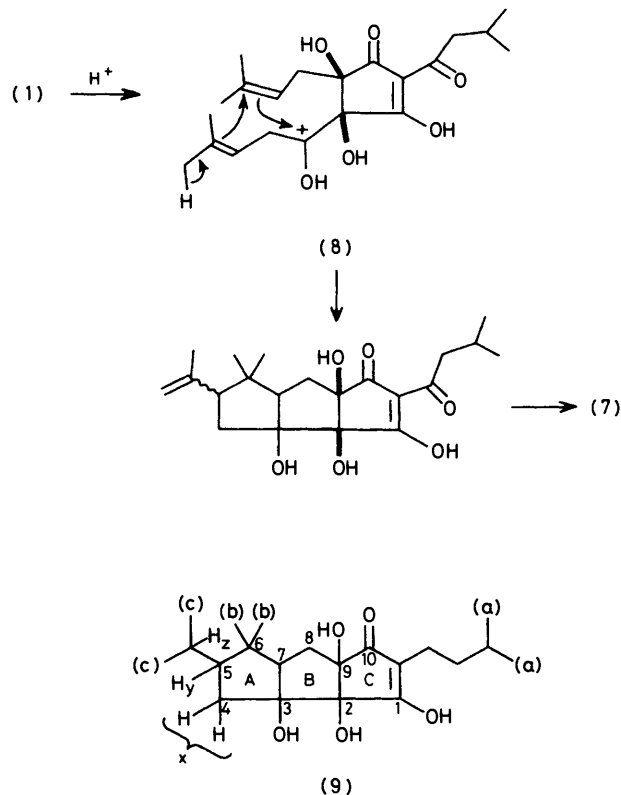
| Chemical shift (p.p.m.) | Multiplicity | Function |
|-------------------------|--------------|-----------------|
| 19.95 | q | CH_3 |
| 22.35 | q | $2 \times CH_3$ |
| 22.61 | q | CH_3 |
| 22.87 | q | CH_3 |
| 24.49 | d | CH |
| 29.37 | q | CH_3 |
| 29.56 | t-(d) | $CH_2(CH)$ |
| 36.32 | t | CH_3 |
| 38.33 | s | C |
| 46.97 | t | CH_2 |
| 61.14 | d-(t) | $CH-(CH_2)$ |
| 82.90 | s | $>C-O$ |
| 86.61 | s | $>C-O$ |
| 87.39 | s | $>C-O$ |
| 113.77 | s | C= |
| 123.77 | s | C= |
| 144.89 | s | C= |
| 197.71 | s | =C-OH |
| 189.23 | s | C=O |
| 199.27 | s | C=O |

Beginning with the retained 5-membered β -tricarboxyl ring system, it was possible from literature analogies^{5,6} to assign the appropriate signals as indicated in the part-structure (6). If the two tertiary hydroxy-groups in humulinone (1) are also retained, then attachment of the necessary further structural elements generates the tricyclic structure (7), which can be rationalised mechanistically as shown in Scheme 1. The general features of



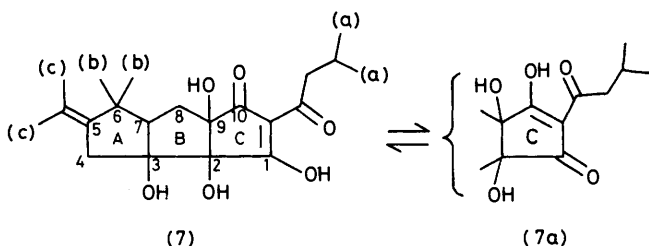
and of the signal from the CH_2 group of the 3-methylbutanoyl side-chain, in the approximate ratio 2 : 1, appeared indicative of the two tautomers (7), (7a). The additional significance of this tautomerism is discussed below.

The original workers³ found that isohumulone A, on hydrogenation over PtO_2 in acetic acid took up an



SCHEME 1

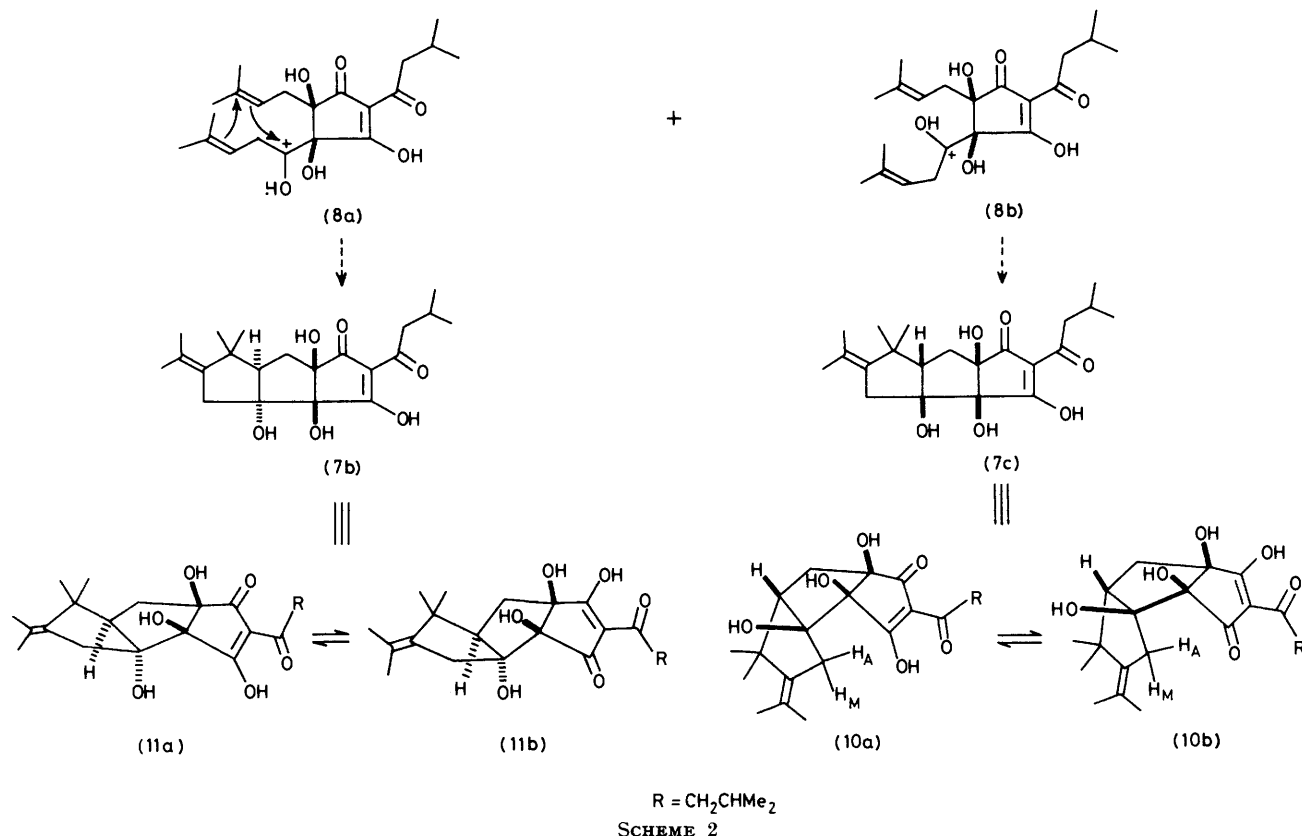
equimolar proportion of hydrogen (in 3 h) to give a dihydroisohumulone A. In our hands a mixture of products was formed but when hydrogenation was allowed to proceed slowly to give an uptake of three molar equivalents of hydrogen, the mixture afforded a crystalline perhydro-derivative $C_{21}H_{34}O_5$ (9). The reduction of both the exocyclic carbonyl^{7,8} and the tetra-substituted double bond^{9,10} have close precedents. In the 400 MHz 1H n.m.r. spectrum of (9), the AMX system of the $CHCH_2$ group of ring B was seen as three double doublets at δ 2.39 (J 14 and 11 Hz), 2.23 (J 14 and 6 Hz), and 1.64 (J 11 and 6 Hz). The geminally coupled protons (x) of ring A in (9), which showed no additional coupling in the spectrum of isohumulone A (7), also appeared as double doublets at δ 1.99 (J 14 and 12 Hz) and 1.38 (J 14 and 6 Hz) in keeping with the extra coupling to the methine proton (y). In the high-field region of the spectrum of (9), the isopropyl methyls (c) showed two doublets (ratio 1 : 1) as expected from their non-equivalence and the coupling to H_z . No additional splitting was evident and this fact, with the appearance of simple double doublets from the protons (x) mentioned



this structure (7) were also supported by the 400 MHz 1H n.m.r. spectrum which was effectively first order. Apart from the expected signals due to the protons of methyl groups (a), (b), and (c) in (7), an AB system for the geminally coupled protons of ring A (J 15.8 Hz), and an AMX system due to the $CHCH_2$ group of ring B (J_{AM} 12.5, J_{AX} 6.5, J_{MX} 13.8 Hz) were evident. The duplication of one half of the AB system, of the AMX system

above, implied the isolation of a single geometrical isomer. The probable mechanism of formation of isohumulone A implies two possible conformations (8a) and (8b) of the presumed intermediate (see Scheme 2), which would be

spectroscopic properties (see Experimental section) closely resemble those of isohumulone A, so it seems probable that the latter is represented by the stereoisomeric structure (7b), *i.e.* (11a and b).



expected to lead to two geometrical isomers of the tricyclic product, (7b) and (7c), the 5-membered rings necessarily being *cis*-fused. Dreiding models indicate that the form (7c) would exist in the folded forms (10a and b), so that hydrogenation of the isopropylidene group would occur on the accessible β -face and be much more stereoselective than with the alternative more 'open' structures (11a and b) which should hydrogenate on both sides of the molecule.

It can also be predicted that the chemical shift of the *endo* geminal hydrogen H_A but not of H_M in structures (10a and b) would be affected by the tautomeric equilibrium whereas there should be no differential effect in the structures (11a and b). Indeed in the 400 MHz ¹H n.m.r. spectrum of isohumulone A (10), proton (M) shows a slightly distorted doublet (total intensity 1 H), and proton (A) shows a triplet (1 H) derivable from the overlap of two similar doublets in the tautomeric ratio 2 : 1.

On the basis of the above results it is suggested that the revised structure for isohumulone A should be (7 \rightleftharpoons 7a) and that its stereostructure can be tentatively represented as the (racemic) tautomers of structure (7c), *i.e.* (10a and b).

As regards isohumulone B, it is noteworthy that the

EXPERIMENTAL

U.v. spectra were recorded on a Unicam SP800 spectrophotometer; i.r. spectra with a Unicam SP200 instrument. ¹H N.m.r. spectra at 400 MHz were measured by the S.R.C. high-field n.m.r. spectroscopic service with a Bruker WH-400 instrument. ¹³C N.m.r. (22.6 MHz) spectra were obtained with respect to tetramethylsilane using a Bruker WH 90 instrument. Mass spectra were recorded with a Varian CH 5D instrument.

Isomerisation of Humulinone (1) to the Isohumulinones A and B.—Humulinone¹¹ (1) (m.p. 71–73 °C; 1.80 g) in benzene (27 ml) was refluxed with formic acid (27 ml) for 1 h and then the solution was cooled to 20 °C. Ether (250 ml) was added to it and the solution washed *five* times with water (5 × 200 ml), dried (MgSO₄), and evaporated (reduced pressure) to yield a gum which was dissolved in hot, light petroleum (b.p. 40–60 °C; 250 ml). Next day a pale orange solid had been deposited (0.552 g). Reduction of the volume of light petroleum (to 50 ml) by evaporation under reduced pressure afforded a second crop of solid (177 mg). Crystallisation of the first crop (from CHCl₃) gave colourless crystals of isohumulone A (217 mg, 12%), m.p. 199–200 °C (lit.,³ 197–198 °C). The second crop was combined with the mother-liquor from the first crop and crystallised from chloroform–light petroleum to give isohumulone B (352 mg, 20%), m.p. 148–150 °C. The

yields were 9 to 16% and 18 to 23% respectively on different occasions.

Isohumulinone A (7c) (from CHCl_3) had m.p. 202–204 °C (Found: C, 66.3; H, 7.93. Calc. for $\text{C}_{21}\text{H}_{30}\text{O}_6$: C, 66.6; H, 7.99%), λ_{max} (acid methanol) 269 (ϵ_{max} 8 700) and 227 (ϵ_{max} 10 600) nm; λ_{max} (alkaline methanol) 273 (ϵ_{max} 15 600), and 253 (ϵ_{max} 18 400) nm; ν_{max} (chloroform) 3 604m, 3 200–3 600br, 2 966m, 2 935m, 2 878m, 1 709s, 1 636s, and 1 586s cm^{-1} ; δ (400 MHz, CDCl_3) 0.87 (6 H, d, J 7 Hz, $\text{COCH}_2\text{CHMe}_2$), 1.21 (3 H, s, >CMe), 1.26 (3 H, s, >CMe), 1.61 and 1.60 (3 H, 2 s, =CMe), 1.762 and 1.757 (3 H, 2 s, =CMe), 1.89 and 1.72 (1 H, 2 dd, each J 12.5, 6.5 Hz, H-8), 2.16 (1 H, m, J 7 Hz, $\text{COCH}_2\text{CHCMe}_2$), 2.22 (1 H, m, $J_{\text{AX}} + J_{\text{MX}}$ 26.5 Hz, H-8), 2.34 (1 H, 2 overlapping d, J 15.5 Hz, 4-H), 2.48 and 2.42 (1 H, 2 dd, each J 14, 6.5 Hz, 7-H), 2.64 (1 H, d, J 15.5 Hz, 4-H), 2.78 (0.66 H, d, J 7 Hz, $\text{COCH}_2\text{CHMe}_2$), 2.80 (1.34 H, m, $\text{COCH}_2\text{CHMe}_2$), 3.45–3.53 (3 H, broad, OH); m/z (%) 378(5), 361(17), 360(78), 346(13), 345(25), 342(7), 327(18), 214(15), 165(10), 164(27), 152(32), 151(100), 149(31), 137(37), 136(13), 135(12), 123(9), 122(19), 121(19), 111(17), 109(10), 105(15), 95(14), 93(14), 91(15), 85(14), 83(14), 81(19), 69(22), 67(19), 57(45), 55(33), 44(20), 43(36), and 41(44).

Isohumulinone B crystallised from chloroform–light petroleum as colourless needles, m.p. 151–153 °C (lit.³ 152 °C) (Found: C, 66.4; H, 7.85. Calc. for $\text{C}_{21}\text{H}_{30}\text{O}_6$: C, 66.6; H, 7.99%), λ_{max} (acid methanol) 268 (ϵ_{max} 9 500), 227 (ϵ_{max} 11 400) nm, λ_{max} (alkaline methanol) 268 (ϵ_{max} 14 400), 254 nm (ϵ_{max} 16 400); ν_{max} (chloroform) (intensity) 3 400b,s, 2 969m, 2 935m, 2 878w, 1 709s, 1 634s, and 1 582s cm^{-1} ; m/z (%) 378(7), 361(24), 360(100), 342(21), 327(15), 258(17), 216(13), 164(47), 151(69), 137(35), 136(30), 135(31), 123(10), 122(10), 121(10), 109(11), 107(10), 105(12), 95(11), 93(12), 91(14), 85(15), 81(15), 79(12), 77(10), 69(12), 67(17), 57(36), 55(19), 44(34), 43(29), and 41(39); δ ^{13}C [$(\text{CD}_3)_2\text{SO}$] 19.96(q), 22.33(q), 23.08(q), 24.69(q), 25.59(d), 28.23(q), 36.02(t-[d]), 42.47(t), 43.38(s), 48.10(t), 63.60(bd), 81.00(s), 82.77(s), 111.66(s), 123.25(s), 140.49(s), 196.46(s), 198.28(s), and 200.15(s); δ (400 MHz, CDCl_3) 0.972 (3 H, d, J 6.5 Hz, $\text{COCH}_2\text{CHMe}_2$), 0.98 (3 H, d, J 6.5 Hz, $\text{COCH}_2\text{CHMe}_2$), 1.17 (3 H, s, >CMe), 1.35 (3 H, s, >CMe), 1.53 (3 H, s, =CMe), 1.71 (3 H, s, =CMe), and 2.36 (1 H, d, J 17.5 Hz, >CH_2). The remaining signals were overlapping multiplets.

Sodium Borohydride Reduction of Photoisohumulone (4).—

(i) *In a two-phase system.* Photoisohumulone (4), m.p. 60–61 °C, prepared as described previously¹² (45 mg) in iso-octane (2 ml) was added to a solution of sodium borohydride (9.40 mg) in water (2 ml) and the mixture stirred vigorously for 22 h at 20 °C. After acidification to pH 1 with 1N-HCl the mixture was extracted with iso-octane (3 × 5 ml). The combined iso-octane extracts were dried (MgSO_4) and evaporated under reduced pressure to give ρ -isohumulones, a mixture of the diastereoisomeric *trans*-compounds (5)¹⁸ (37 mg, 82%). The compounds gave R_F values of 0.25 and 0.46 in formic acid–ethyl formate–hexane (1 : 8 : 12) (R_F of isohumulone = 0.37), λ_{max} (acid ethanol) 279 and 224 nm; λ_{max} (alkaline ethanol) 272 and 252 nm; ν_{max} (chloroform) (intensity) 3 530br, m, 2 958m, 2 921m, 2 873w, 1 691w, 1 635s, and 1 587s cm^{-1} ; δ (90 MHz, CDCl_3) 0.07 (6 H, d, J 6.5 Hz, $\text{COCH}_2\text{CHMe}_2$), 1.58, 1.53, and 1.70 (12 H, 3 s, 2 × C=CMe₂), 1.8–3.0 [7 H, multiplets, $\text{CHCH}_2\text{CH}=\text{CMe}_2$, $\text{CH(OH)CH}_2\text{CH}=\text{CMe}_2$, and $\text{COCH}_2\text{CHMe}_2$], 2.77 (2 H, d, J Hz, $\text{COCH}_2\text{CHMe}_2$), 3.78 (1 H, m, CHOH), 5.2 (2 H, m, 2 × CH=CMe₂), and 6.5br (OH); m/z (%) 364(5),

346(18), 277(18), 266(12), 235(9), 210(15), 197(100), and 109(27).

(ii) *In a single-phase system.* A solution of photoisohumulone (4) (10 mg) and sodium borohydride (2 mg) in ethanol–water (9 : 1; 1 ml) was stirred at 20 °C and the reaction followed by t.l.c.; the latter indicated that the reaction was complete within 1½ h. Dilute hydrochloric acid was added to the mixture and the products were extracted into chloroform. The chloroform layer was washed with water, dried (MgSO_4), and evaporated under reduced pressure to yield an oil (8.5 mg, 90%) which ^1H n.m.r. and i.r. spectroscopy and t.l.c. showed to be a mixture of the ρ -isohumulones (5) as in the two-phase reaction above.

Sodium Borohydride Reduction of Humulinone (1).—Humulinone (120 mg) and sodium borohydride (25 mg) were dissolved in ethanol–water (9 : 1, 12 ml) and the solution was stirred at 20 °C for 1½ h. Water (20 ml) was added to the mixture and, after acidification to pH 1, the solution was extracted with chloroform (20 ml), and the extract dried (MgSO_4) and evaporated under reduced pressure to yield an oil (114 mg). In the ^1H n.m.r. spectrum of the latter measurement of the doublet at δ 3.4 ($\text{COCH}_2\text{CH}=\text{CMe}_2$ of humulinone) and the multiplet at δ 3.85 (CHOH of ρ -humulinone) (cf. ref. 13) indicated ca. 65% conversion of the former into the latter. The oil (114 mg) was treated with fresh sodium borohydride (25 mg) for a further 2½ h and worked up as before. The ^1H n.m.r. spectrum now indicated ca. 85% conversion into ρ -humulinone. Repetition of the reduction with fresh borohydride (25 mg) and work-up as before after a further 12 h gave ρ -humulinone free from humulinone, as an oil (87 mg, 76%), λ_{max} (acid ethanol) 282 and 228 nm; λ_{max} (alkaline ethanol) 272 and 258 nm; δ (CDCl_3) 0.96 (6 H, d, J 6 Hz, $\text{COCH}_2\text{CHMe}_2$), 1.59 and 1.69 (12 H, 2 s, 2 × CH=CMe₂), 1.8–2.9 (series of overlapping multiplets), 3.8 (1 H, m, CHOH), 3.3–5.5br (OH), and 5.2 (2 H, m, 2 × CH=CMe₂).

Sodium Borohydride Reduction of Isohumulinone A (7c).—Isohumulinone A (21 mg) and sodium borohydride (6.1 mg) were dissolved in ethanol–water (9 : 1; 2.7 ml) and stirred at 20 °C for 12 h. Water (10 ml) was added to the mixture and the solution acidified to pH 1 (dilute HCl). The mixture was extracted with chloroform (10 ml), and the extract washed with water, dried (MgSO_4), and evaporated under reduced pressure to yield a solid. The ^1H n.m.r. spectrum of the latter was identical with that of isohumulone A (7c). Recrystallisation of the product from chloroform afforded a colourless solid, m.p. 197–197.5 °C, mixed m.p. 194–199 °C with pure isohumulone A (m.p. 199–201 °C).

Hydrogenation of Isohumulinone A (7c).—(i) *1 Mol equiv. of hydrogen.* Isohumulinone A (30.4 mg) in acetic acid (2 ml) was hydrogenated over platinum oxide (5.0 mg) and allowed to consume 1 mol equiv. of hydrogen (35 min). The solution was filtered and the filtrate evaporated under reduced pressure to give a colourless gum. Trituration with boiling chloroform afforded a colourless solid (10.7 mg), m.p. 189–193 °C (decomp.). Mixed m.p. 184–187 °C with pure isohumulone A of m.p. 201–203.5 °C. T.l.c. and ^1H n.m.r. and mass spectrometry indicated that the solid was a mixture of compounds.

(ii) *2 Mol equiv. of hydrogen.* Isohumulinone A was hydrogenated as above and allowed to consume 2 mol equiv. of hydrogen (65 min). The product was a mixture similar to the mixture produced above.

(iii) *3 Mol equiv. of hydrogen.* Isohumulinone A (55 mg) in

acetic acid (3.6 ml) was hydrogenated over platinum oxide (9.1 mg) and allowed to consume 3 molequiv. of hydrogen (3 h). The solution was filtered and the filtrate evaporated under reduced pressure to give a colourless gum. Trituration with boiling chloroform yielded a colourless solid (32 mg), m.p. 172—183 °C (decomp.). Two recrystallisations of this from methanol-water afforded the *dihydrodeoxoisohumulone A* (10), m.p. 205—210 °C (decomp.), λ_{\max} (ethanol) 256 nm (ϵ_{\max} 12 400); λ_{\max} (alkaline ethanol) 277 nm (ϵ_{\max} 20 500); ν_{\max} (Nujol mull) (intensity) 3 440m, 1 660w, and 1 588s; δ (400 MHz, CD₃OD) 0.90 (6 H, d, J 6.5 Hz, CH₂CH₂CHMe₂), 0.98 and 0.92 (6 H, 2 d, each J 6.5 Hz, CH·CHMe₂), 1.07 (3 H, s, CMe), 1.08 (3 H, s, >CMe), 1.29 (2 H, m, CH₂-CH₂CHMe₂), 1.38 (1 H, dd, J 14, 6 Hz, 4-H), 1.56 (1 H, dd, J 12, 6 Hz, H-5), 1.6—1.5 (1 H, obscured m, CH₂CH₂CHMe₂), 1.64 (1 H, dd, J 11, 6 Hz, 7-H), 1.74 (1 H, m, CH·CHMe₂), 1.08 (1 H, dd, J 14, 12 Hz, 4-H), 2.08 (2 H, m, CH₂CH₂-CHMe₂), 2.23 (1 H, dd, J 14, 6 Hz, 8-H), 2.39 (1 H, dd, J 14, 11 Hz, 8-H); m/z (%) 366(2), 348(53), 334(45), 333(100), 315(14), 291(19), 279(19), 195(15), 167(17), 151(19), 139(92), 55(28), and 43(42) (Found: M^+ , 366.2428. C₂₁H₃₄O₅ requires M , 366.2406).

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