

Oxidation and Isomerisation of Colupulone; the Separation and Structures of Two Transformation Products

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Colupulone (2) when boiled under aerobic conditions in acid (pH 5.4) buffer is transformed in good yield to, amongst other products, two non-polar tricyclic ketones, 8-hydroxy-4,4-dimethyl-3-isopropyl-7-(3-methylbut-2-enyl)-9-(2-methylpropionyl)tricyclo[5,3,1,0^{4,5}]undecane-10,11-dione (12) and its didehydro-derivative (13), separable by silver nitrate-silicic acid chromatography. The minor product (13) may be obtained more conveniently by treatment of colupulone (2) with lead tetra-acetate.

THE hop constituents the lupulones (1) and, in particular, the homologue colupulone (2) are assumed to contribute relatively little to the composition of the aqueous solution (pH *ca.* 5.2) which results from boiling hops with sweet wort in the brewing process. It is known, however, that oxidation and rearrangement of colupulone (2) gives cohulupone (3),¹ whilst boiling in strongly acid or alkaline aqueous solutions causes degradation to structures, such as (4)² and (5)³ respectively.

Because of the relevance of more moderate pH conditions to the brewing procedure, we examined the course of the reaction when colupulone (2) was boiled in an aqueous buffer solution at pH 5.4 open to the air.

Two products appeared to be formed during the first hour of which one, as judged by t.l.c., was of unusually low polarity. Column chromatography afforded this product in good yield (37%) as a pale yellow oil.

The field desorption (f.d.) mass spectrum⁴ showed only two high abundance ions at *m/e* 400 and 398, and these were later confirmed by the electron impact (e.i.) mass spectrum as *two* molecular ions, 400 (100%) and 398 (36%) corresponding to C₂₅H₃₆O₄ and C₂₅H₃₄O₄ respectively. It proved impossible to separate this material by t.l.c. in ordinary systems, but on hydrogenation over palladised charcoal it consistently took up more hydrogen than required for C₂₅H₃₆O₄, in keeping with the existence of a minor amount of a compound C₂₅H₃₄O₄.

The u.v. spectrum, λ_{max.} 235 and 280 nm, λ_{max.}(alkali) 248sh, 270, and 282sh nm, was quite different from colupulone (2) and very similar to that of colupone (6)⁶

¹ D. Wright, *J. Chem. Soc.*, 1963, 1769.

² G. A. Howard, J. R. A. Pollock, and A. R. Tatchell, *J. Chem. Soc.*, 1955, 174.

³ J. P. Regan and J. A. Elvidge, *J. Inst. Brewing*, 1969, **75**, 10.

⁴ H. D. Beckey and H. R. Schulten, *Angew. Chem. Internat. Edn.*, 1975, **14**, 403.

⁵ F. Alderweireldt and M. Verzele, *Bull. Soc. chim. belges*, 1957, **66**, 391.

and other tetrasubstituted acylcyclohexane-2,4,6-tri-ones.⁷ The presence of the six-membered ring was further evident from the low-field region of the ¹H n.m.r. spectrum which showed, at 35 °C, two barely separated broad singlets at τ -8.72 and -8.80 (together 1 H). At 55 °C these signals coalesced whilst at -20 °C they became completely resolved, facts which were consistent with chelation of the isobutyryl side-chain carbonyl group with two alternative enol groups as shown in (7).

The i.r. spectrum of the mixture showed, besides the expected carbonyl absorption of the β-triketone system, a strong band at 1750 cm⁻¹. It therefore appeared that the C-5 carbonyl group, unlike that in colupone (6) (at 1700 cm⁻¹)⁶ or leptospermone (8)⁷ (at 1715 cm⁻¹), was part of a five-membered ring system. Formation of a free radical at C-6 as in (9) in colupulone (2) is very likely and has previously been implied to explain, by oxidative coupling, the formation of a dimer,⁸ from its reaction (and that of a model compound⁹) with lead tetra-acetate. These facts and the observation that colupulone was unchanged when boiled in the buffer solution under nitrogen led us to propose the cyclisation (9)→(10) shown in the Scheme to generate the five-membered ring ketone system. The remaining cyclisation, (10)→(11), leading to the main product (12) C₂₅H₃₆O₄, is required by the presence in the ¹H n.m.r. spectrum of signals assignable only to one isopropyl and one *gem*-dimethyl group (multiplet τ 8.95—9.12) in addition to the well known isobutyryl and dimethylallyl signals of the C-2 and C-4 substituents respectively. Dreiding models show that the formation of (12) is

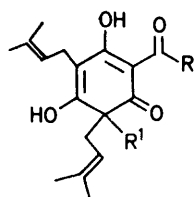
⁶ D. R. J. Laws, *J. Chem. Soc.*, 1965, 6542.

⁷ W. R. Chan and C. H. Hassall, *J. Chem. Soc.*, 1956, 3495.

⁸ M. Collins, D. R. J. Laws, J. D. McGuinness, and J. A. Elvidge, *J. Chem. Soc.*, 1971, 3817.

⁹ P. R. Ashurst, P. M. Brown, J. A. Elvidge, and R. Stevens, *J. Chem. Soc.*, 1965, 6543.

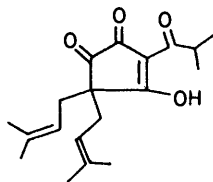
sterically feasible, whilst the cyclisations of (10) or (10a), shown in dotted lines, which might lead to isomeric compounds containing the same functional groups, are



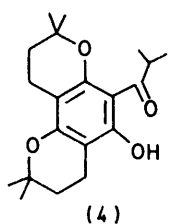
(1) $R^1 = \text{CH}_2\text{CH}:\text{CMe}_2$

(2) $R = \text{CHMe}_2, R^1 = \text{CH}_2\text{CH}:\text{CMe}_2$

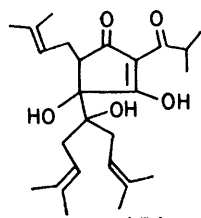
(15) $R = \text{CH}_2\text{CHMe}_2, R^1 = \alpha\text{-OH}$



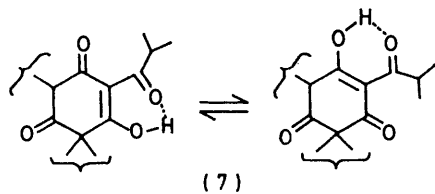
(3)



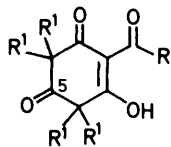
(4)



(5)

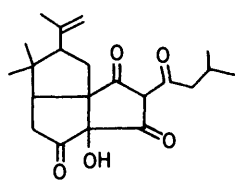


(7)



(6) $R = \text{CHMe}_2, R^1 = \text{CH}_2\text{CH}:\text{CMe}_2$

(8) $R = \text{CH}_2\text{CHMe}_2, R^1 = \text{Me}$



(14)

sterically impossible. Loss of hydrogen from (11) would afford the dehydro-derivative (13), $\text{C}_{25}\text{H}_{34}\text{O}_4$, of predictably very similar chromatographic properties to (12), which we anticipated was the minor component in the mixture. In fact the ^1H n.m.r. spectrum showed, in addition to the signals expected from (12), two broad signals (each *ca.* 0.25 H) at τ 5.02 and 5.20 and a distinct shoulder at τ 8.25, assignable to the vinyl and methyl hydrogens of the isopropenyl grouping of *ca.* 25% of (13).

The product of hydrogenation of this mixture was an oil of very similar R_F to the starting mixture. It showed in its ^1H n.m.r. spectrum the complete disappearance of the vinylic hydrogens and methyl groups and in the mass spectrum gave a molecular ion at m/e 402 a.m.u. (87%) and virtually no ion current at m/e 400 (5%) and 398 (< 1%).

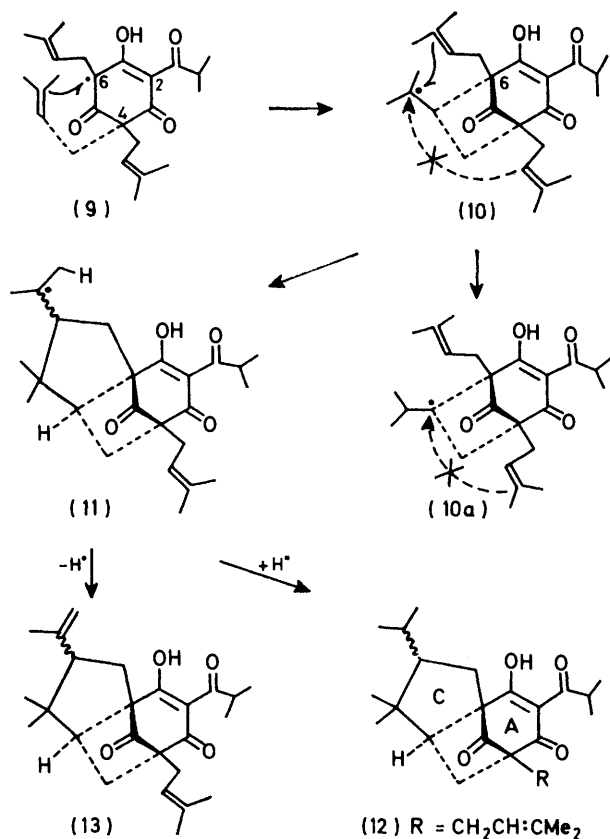
Separation of the original mixture of (12) and (13) was achieved by careful chromatography on silicic acid impregnated with 25% of silver nitrate. The two

components (12) and (13) were obtained in the expected ratio of 3 : 1 respectively.

The major component (12) showed in its ^1H n.m.r. spectrum (at 90 MHz) the absence of the isopropenyl group signals of (13) mentioned above and a singlet at τ 9.02 superimposed on a just resolved doublet, $J \sim 7$ Hz, assignable to the *gem* dimethyl and isopropyl groups of ring c respectively, and no signals at higher field.

The minor component (13) was in fact, as expected, produced in much better yield, along with other products, by treatment of colupulone (2) with lead tetra-acetate. The sample thus obtained, identical to that formed by aerobic aqueous boiling, showed the vinyl hydrogens as two broad singlets in the ^1H n.m.r. spectrum as above, each of intensity 1 H, and the vinyl methyl as a singlet (3 H), τ 8.23, in addition to the dimethylallyl singlet at τ 8.32. In the high-field region, the *gem*-dimethyl group appeared as two singlets at τ 9.04 and 9.45. This contrasts strongly with the spectrum of (12) and may be explained by the shielding of one of the methyl groups by the isopropenyl double bond.

The e.i. mass spectra of both (12) and (13) showed abundant molecular ions (98 and 100%, respectively) and



SCHEME

each showed the expected fragment ions at m/e 69 and 71 a.m.u. as well as two ions at $M^+ - 68$ and $M^+ - 69$ corresponding to loss of a dimethylallyl group with and without hydrogen rearrangement. In the fragmentation

pattern at high m/e values, the main difference could be explained by the substitution of the isopropenyl group in (13) for the isopropyl group in (12). Thus (12) showed two ions at m/e 358 (23%) and 357 (17%) corresponding to loss of the isopropyl group with and without hydrogen rearrangement, whereas (13) showed only one ion, m/e 358 (17%), corresponding to loss of the isopropenyl group. This fragmentation of the isopropenyl group from (13) (-40 a.m.u.) was also indicated by ions at m/e 315 (35%) and 275 (39%); (12) showed no equivalent ion near m/e 315 but also gave an abundant ion (65%) at m/e 275.

The stereo-structures of (12) and (13), which must be racemic, follow almost completely from the steric constraints on the construction (a) of the *cis* bridged ring system and (b) of the *cis* fused five-membered **b** and **c** rings. The configuration of the isopropyl [in (12)] and isopropenyl [in (13)] groups are, however, uncertain since the closure of ring **c** [(10) \rightarrow (11 in the Scheme)] could, in principle, occur on either face of the 6-dimethylallyl group in (10).

The cyclisation of the dimethylallyl groups evident in the formation of these two tricyclic derivatives parallel those which take place in the formation of tricyclic dehydroisohumulone¹⁰ (14) when humulone (15) is treated with lead tetra-acetate, but in this reaction ring contraction of the humulone ring system also takes place. Since the original investigation¹⁰ of the structure of tricyclic dehydroisohumulone, recently revised¹¹ to (14), it has been shown that (14) can also be formed during aerobic boiling¹¹ of humulone (15) in water. It is, therefore, probable that this type of double cyclisation is important in the fate of both humulones and lupulones during brewing.

EXPERIMENTAL

General experimental conditions were as in ref. 12.

Boiling of Colupulone in Buffer.—Colupulone (2), m.p. 92–94 °C (1.0 g) was heated to reflux in phosphate buffer solution (1 l; pH 5.4) made up from Na_2HPO_4 4.35 g l⁻¹; KH_2PO_4 8.24 g l⁻¹, adjusted to pH 5.4 with H_3PO_4 for 1½ h. The mixture was acidified (4M-HCl) and extracted into chloroform (2 × 100 ml). After drying ($\text{MgSO}_4 \cdot \text{H}_2\text{O}$) the solvent was evaporated under reduced pressure to leave a brown oil (0.80 g).

Chromatography on silicic acid (Mallinckrodt 100 mesh, column 26 × 3 cm) and elution with diethyl ether–light petroleum (b.p. 40–60 °C) (1:9) afforded a yellow oil (373 mg). P.l.c. gave a mixture of (12) and (13) (280 mg), R_F 0.64 (5% EtOAc–light petroleum, b.p. 40–60 °C), λ_{max} 235 (ethanol) and 280 nm; λ_{max} (alkaline ethanol) 248sh, 270, and 282sh nm; ν_{max} (CHCl_3) 1 750, 1 660, and 900 cm⁻¹; $\tau(\text{CDCl}_3)$ 9.04 (d, J ca. 7 Hz, partially obscured, CHCMe_2), 9.02 (s, CMe_2) 8.86, 8.84, (2 d, each J 7 Hz, $\text{CO}\cdot\text{CHMe}_2$), 8.33 (s, :CMe_2), 8.23 (s, $\text{CH}_2\cdot\text{CMe}$), 7.55–8.15 (m, 2 × CH_2), 7.42 (m, $\text{CH}_2\cdot\text{CH}$), 5.87 and 5.84 (2 m, J 7 Hz, $\text{CO}\cdot\text{CHMe}_2$), 5.20br and 5.02br (2s, :CH_2), 4.76br

(t, J ca. 7 Hz, $\text{C}\cdot\text{CH}$), and -8.71br and -8.80br (2 s, chelated OH); m/e (f.d.) 400 (100%) and 398 (87%); m/e (e.i.) (%) 400 (100), 398 (36), 382 (18), 372 (13), 358 (56), 357 (16), 343 (18), 332 (22), 331 (24), 330 (23), 329 (22), 315 (27), 275 (57), 261 (26), 259 (22), 257 (29), 234 (29), 219 (28), 163 (28), 123 (23), 121 (22), 107 (22), 105 (21), 95 (41), 93 (23), 91 (32), 81 (20), 79 (29), 78 (28), 77 (28), 71 (57), 69 (96), 67 (34), 54 (51), and 52 (27).

Hydrogenation.—The above mixture of ketones (12) and (13) (130 mg) in ethanol (5.0 ml) was hydrogenated over 10% palladised charcoal (25 mg) at 20 °C and atmospheric pressure. After the uptake of hydrogen (8.6 ml) had ceased, the catalyst was filtered off and the solvent evaporated under reduced pressure. The resulting oil was chromatographed on silicic acid (as above, but 22 × 2.0 cm column) and elution with diethyl ether–light petroleum (b.p. 40–60 °C) (1:9) afforded the *dihydro-derivative* (12; $R = \text{CH}_2\text{CH}_2\text{CHMe}_2$), as a pale yellow oil (112 mg), R_F 0.65 (system as above); λ_{max} (ethanol) 235 (ϵ 8 100) and 280 nm (13 230) nm; λ_{max} (alkaline ethanol) 245sh. (6 980), 272 (15 380), and 285sh nm (13 120); ν_{max} (CHCl_3) 1 750, 1 660, and 1 555 cm⁻¹; $\tau(\text{CDCl}_3)$ 9.06 (d, J ca. 7 Hz, 2 × CHMe_2), 9.04 (s, CMe_2), 8.87 and 8.85 (2d, each 7 Hz, COCHMe_2), 7.6–8.7 (series of m, CH_2 and CH), 5.95 (m, COCHMe_2), and -9.16br and -9.30br (2s, chelated OH); m/e (f.d.) 403 (100%) and 361 (44%); m/e (e.i.) (%) 402 (M^+ , 87), 384 (10), 360 (17), 359 (13), 341 (15), 331 (13), 305 (56), 292 (14), 291 (32), 290 (14), 287 (21), 277 (18), 263 (85), 245 (11), 235 (13), 221 (18), 205 (11), 193 (15), 165 (18), 163 (12), 149 (16), 137 (17), 135 (14), 123 (19), 121 (18), 109 (31), 107 (22), 105 (17), 98 (20), 97 (30), 95 (30), 93 (19), 91 (30), 83 (29), 81 (23), 79 (24), 77 (17), 71 (65), 69 (73), 67 (22), 57 (13), 55 (68), 43 (100), and 41 (87) (Found: C, 74.7; H, 9.28%; M^+ , 4 02.277 4. $\text{C}_{25}\text{H}_{36}\text{O}_4$ requires C, 74.6; H, 9.52%; M , 402.277 0).

Separation of the Ketones (12) and (13).—The mixture of (12) and (13) (175 mg) was chromatographed on silicic acid (as above) impregnated with silver nitrate (25%). Elution with diethyl ether–light petroleum (1:40) afforded 8-hydroxy-3-isopropyl-4,4-dimethyl-7-(3-methylbut-2-enyl)-9-(2-methylpropionyl)tricyclo[5.3.1.0^{1,5}]undecane-10,11-dione (12) as a pale yellow oil (120 mg), R_F 0.64 in the above mentioned system, λ_{max} (ethanol) 235 (ϵ 7 640) and 280 nm (13 330); λ_{max} (alkaline ethanol) 245sh (7 530) 270 (15 500), and 282sh nm (13 330); ν_{max} (CHCl_3) 1 750, 1 660, and 1 550 cm⁻¹; $\tau(\text{CDCl}_3)$ 9.04 (partially obscured d, J ca. 7 Hz, CHMe_2), 9.02 (s, CMe_2), 8.86 and 8.84 (6 H, 2d, each J 7 Hz, $\text{CO}\cdot\text{CHMe}_2$), 8.33 (6 H, s, :CMe_2), 8.2–7.5 (m, CH_2), 7.4 (m, $\text{CH}_2\cdot\text{CMe}_2$), 5.7–6.2 (1 H, m, $\text{CO}\cdot\text{CHMe}_2$), 4.76 (1 H, m, $\text{CH}\cdot\text{CMe}_2$) and -8.73br and -8.80br (total 1 H, 2s, chelated OH); m/e (e.i.) (%) 400 (M^+ , 98), 382 (24), 358 (17), 357 (23), 339 (19), 332 (41), 331 (40), 289 (14), 275 (65), 261 (42), 257 (28), 246 (18), 234 (26), 219 (21), 205 (22), 177 (14), 163 (22), 149 (16), 135 (17), 121 (19), 107 (18), 105 (18), 97 (20), 95 (35), 91 (22), 79 (22), 77 (19), 71 (66), 69 (94), 67 (24), 57 (17), 55 (47), 53 (17), and 42 (100) (Found: M^+ , 400.261 9. $\text{C}_{25}\text{H}_{36}\text{O}_4$ requires M , 400.261 3).

Elution with diethyl ether–light petroleum (b.p. 40–60 °C) (3:17) afforded 8-hydroxy-4,4-dimethyl-7-(3-methylbut-2-enyl)-3-(1-methylvinyl)-9-(2-methylpropionyl)tricyclo[5.3.1.0^{1,5}]undecane-10,11-dione (13) as a pale yellow oil (40 mg) (Found: M^+ , 398.245 1. $\text{C}_{25}\text{H}_{34}\text{O}_4$ requires M^+ , 398.245 7). The compound was identical in t.l.c. in several

¹⁰ J. A. Elvidge, D. R. J. Laws, J. D. McGuinness, and P. V. R. Shannon, *Chem. and Ind.*, 1974, 573.

¹¹ A.-M. Davis, J. A. Elvidge, D. R. J. Laws, J. D. McGuinness, and P. V. R. Shannon, *J.C.S. Perkin I*, in the press.

¹² P. V. R. Shannon and G. D. John, *J.C.S. Perkin I*, 1977, 2585.

systems (R_F values 0.6—0.9) and its n.m.r. and i.r. spectra with a sample prepared as described below.

Oxidation of Colupulone (2) with Lead Tetra-acetate.—Colupulone (1.0 g), lead tetra-acetate (6 g), and glacial acetic acid (40 ml) was kept at 20 °C for h. Water (100 ml) was added and the mixture was extracted with light petroleum (b.p. 60—80 °C) (2 × 50 ml) and the extract dried ($MgSO_4 \cdot H_2O$). The solvent was evaporated under reduced pressure to leave a yellow oil (927 mg). Chromatography on silicic acid (as above, column 24 × 2 cm) and elution with light petroleum (b.p. 40—60 °C) gave an oil (138 mg) which was purified by silver nitrate chromatography as described above. The ketone (13) was obtained as a very pale yellow oil (84 mg), λ_{max} (ethanol) 236 (ϵ 7 560) and 280 nm (12 340); λ_{max} (alkaline ethanol) 245sh (7 300), 270 (14 860), and 283sh nm (13 010); ν_{max} ($CHCl_3$) 1 750, 1 660, 1 555, and 900 cm^{-1} ; $\tau(CDCl_3)$ 9.45 and 9.04 (6 H,

2s, CMe_2), 8.86 and 8.84 (6 H, 2d, each J 7 Hz, $CO-CHMe_2$), 8.32 (6 H, s, $:CMe_2$), and 8.24 (3 H, s, $CH_2:CMe$), 8.13—7.2 (series of m, CH_2 and CH), 5.97 and 5.94 (1 H, 2m, each J 7 Hz, $CO-CHMe_2$), 5.2br and 5.02br (2 H, 2s, $C:CH_2$), 4.78 (1 H, m, $Me_2C:CH$), and -8.70br and -8.81br (1 H, 2 s, chelated OH); m/e (f.d.) 398 (100%), m/e (e.i.) (%) 398 (M^+ , 100), 382 (16), 380 (30), 370 (23), 355 (25), 337 (31), 331 (13), 330 (58), 329 (78), 315 (21), 311 (26), 301 (35), 287 (32), 275 (85), 259 (65), 257 (29), 247 (37), 246 (32), 241 (54), 234 (49), 233 (25), 219 (20), 217 (33), 203 (35), 163 (34), 161 (45), 149 (39), 147 (31), 135 (32), 133 (25), 123 (22), 121 (58), 119 (32), 109 (37), 107 (42), 105 (47), 97 (26), 95 (62), 94 (43), 91 (58), 83 (31), 81 (44), 79 (48), 77 (45), 71 (85), 69 (94), 67 (56), 58 (23), 56 (84), and 54 (41).

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