Chemistry of Hop Constituents. Part 41.[1] Tricyclodehydroisohumulone

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Tricyclodehydroisohumulone, detected as a new bittering component present in beer and in stored hops, is formed in low yield by boiling aqueous humulone in air. The new compound is best prepared (30%) by dehydrogenation of humulone with lead tetra-acetate in a slurry of acetic acid at 0 °C. Spectroscopic, mass spectrometric, and chemical studies suggest that the compound is 5-hydroxy-9,9-dimethyl-3-(3-methylbutanoyl)-10-(1-methyl-ethenyl)tricyclo[6.3.0^{1,5}.0^{1,8}]undeca-2,4,6-trione. Evidence for the probable absolute configuration is discussed.

DURING storage of hops, the humulone content can decrease rapidly without there being a corresponding fall in bittering potential.[2] A study of aspects of this

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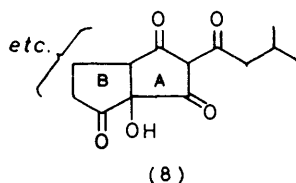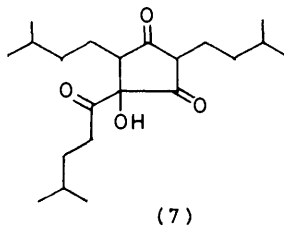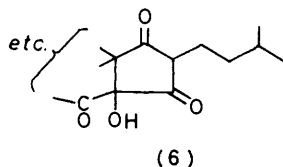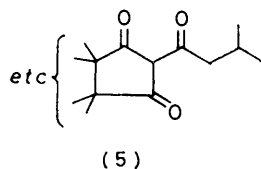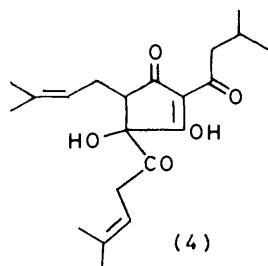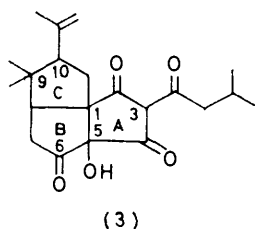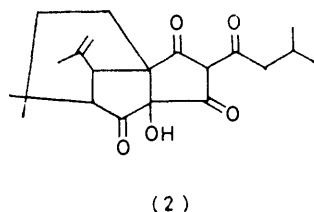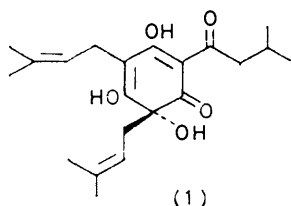
phenomenon led to the recognition of a new compound in stored hops (up to 0.3%) and in beers (up to 4 p.p.m.), which was identical with a product from dehydrogen-

ation of humulone (1) with lead tetra-acetate. The same compound was also obtained (in low yield) by boiling an aqueous solution of humulone in the presence of air. Preliminary chemical and spectroscopic results led us to propose a fused 5,5,6-tricyclic ring structure (2) for the new compound which we named tricyclodehydroiso-humulone.[3] We now present a full account of the work, together with recent results which necessitate a revision of the structure to the fused 5,5,5-tricyclic system (3).

A solution of humulone (1) in acetic acid was cooled to 0 °C and the slurry stirred with an excess of lead tetra-acetate (1.6 molar proportions). Subsequent dilution with water and extraction with ether afforded the product, m.p. 171 °C (decomp.), as a crystalline solvate with acetic acid. Sublimation, or shaking with water, then provided tricyclodehydroisohumulone (3), m.p. 163 °C, $[\alpha]_D^{20}$ +299° (MeOH). Of several variants, this procedure for the oxidation gave consistent yields (*ca.* 30%). When conducted similarly in propionic acid, the oxidation gave tricyclodehydroisohumulone as a crystal-line solvate with propionic acid.

From the elementary composition $C_{21}H_{28}O_5$ and mass-spectral molecular weight (360), tricyclodehydroiso-humulone was evidently a monomeric dehydrogenation product of humulone ($C_{21}H_{30}O_5$). The presence of a 5-membered ring β-tricarbonyl system, as in the iso-humulones [4,5] (4), was strongly indicated by the u.v. absorption maxima at 237 and 268 nm in acidified ethanol and the change to $\lambda_{max.}$ 257 nm in alkaline ethanol. Independent evidence for the β-tricarbonyl feature (5) came from the mass spectrum of tricyclo-dehydroisohumulone which showed a major fragment-ation, m/e 360 $\longrightarrow m/e$ 207, involving loss of $C_8H_9O_3$ (as determined by accurate mass measurement). The [1]H n.m.r. spectrum of tricyclodehydroisohumulone showed the characteristic methylene and split gem-dimethyl signals of the 3-methylbutanoyl side-chain [as in (5)], and also demonstrated the presence of an isopropenyl group $CH_2=C(Me)$, and of a gem-dimethyl group attached to quaternary carbon. One of the last methyls was strongly shielded (τ 9.46), suggesting *e.g.* that it was held closely over the framework of the rest of the molecule. Another fact was the absence of any low-field ^{1}H signal attributable to a bonded enolic hydroxy, a contrast to humulone,[6] lupulone,[7] or isohumulone.[4] The apparent inhibition of enolisation or merely absence of strong

chelation in the β-tricarbonyl system (5) (at least in deuteriochloroform) probably resulted from strain in the 5-membered ring caused by its fusion to a second such ring. The ¹H n.m.r. spectrum thus showed that the β-tricarbonyl system was not part of a 6-membered ring, in conformity with the indications from the u.v. results. However, the system (5) behaved fully as expected towards hydrogenation over Adams' catalyst, the exocyclic carbonyl group being reduced to a saturated methylene group:[8,9] the isopropenyl function was also reduced. The nature of the product, as (6), was revealed by the composition $C_{21}H_{32}O_4$ and the ¹H n.m.r. spectrum, and was confirmed by the similarity of the u.v. spectrum to that of neohydroisohumulone[17] (7) both in acidic ethanol and in alkaline ethanol. The i.r. spectrum of tricyclodehydroisohumulone showed bands (amongst others) clearly attributable to a tertiary hydroxy-group, a 5-membered cycloalkanone (1 760 cm⁻¹),[11] and a vinylic methylene group.

These various findings strongly suggested for tricyclodehydroisohumulone a structure based on the 5,5-bicyclic system (8), derivable formally by dehydrogenation from isohumulone (4). The remainder of the structure (8) had necessarily to be completed with another ring c together with the gem-dimethyl and isopropenyl groups. The range of possibilities was narrowed to structures (2) and (3) by recourse to ¹³C n.m.r. spectroscopy. Comparison of the ¹³C n.m.r. spectra obtained with and without proton spin decoupling revealed or confirmed the presence of the functions listed in the Table. The singlet resonance at δ 108.5, necessarily

analogous carbon atom in e.g. isohumulone and humulinic acid.[12] One or other of the ring 2- or 4-carbonyl groups (which gave the signals at δ 191.3 and 199.1) was enolised or was participating in rapid tautomerism, in line with the u.v. observations, but the enol hydrogen was evidently not strongly chelated, as already mentioned.

A distinction in favour of the structure (3) for tricyclo-dehydroisohumulone was eventually achieved through degradation with periodate. By analogy with humulinic acid C, cleavage of the β-tricarbonyl system (5) was to be expected, with loss of the acyl side-chain.[9] Initial experiments showed that oxidation with periodate occurred only very slowly, suggesting that the course of the reaction was different in this case possibly because of the apparently different enolic character of the ring A in tricyclodehydroisohumulone. From the reaction mixture by t.l.c. on fine silicic acid, a product $C_{20}H_{28}O_4$ (m/e 332.1986) was isolated, which corresponded to oxidative cleavage of ring A in tricyclodehydroisohumulone (3) with loss of one carbonyl group as carbon dioxide but no loss of the original acyl side-chain. In agreement, the mass spectrum of the product showed fragment ions at 247 and 205 a.m.u. corresponding to progressive loss of C_5H_9O and then C_2H_2O from the acyl group, now a β-dicarbonyl side-chain. The u.v. absorption ($\lambda_{max.}$ 277 nm), reminiscent of that of acetylacetone,[13] indicated that the new side-chain was enolised. Structures (9) and (10) were consistent with these findings, but
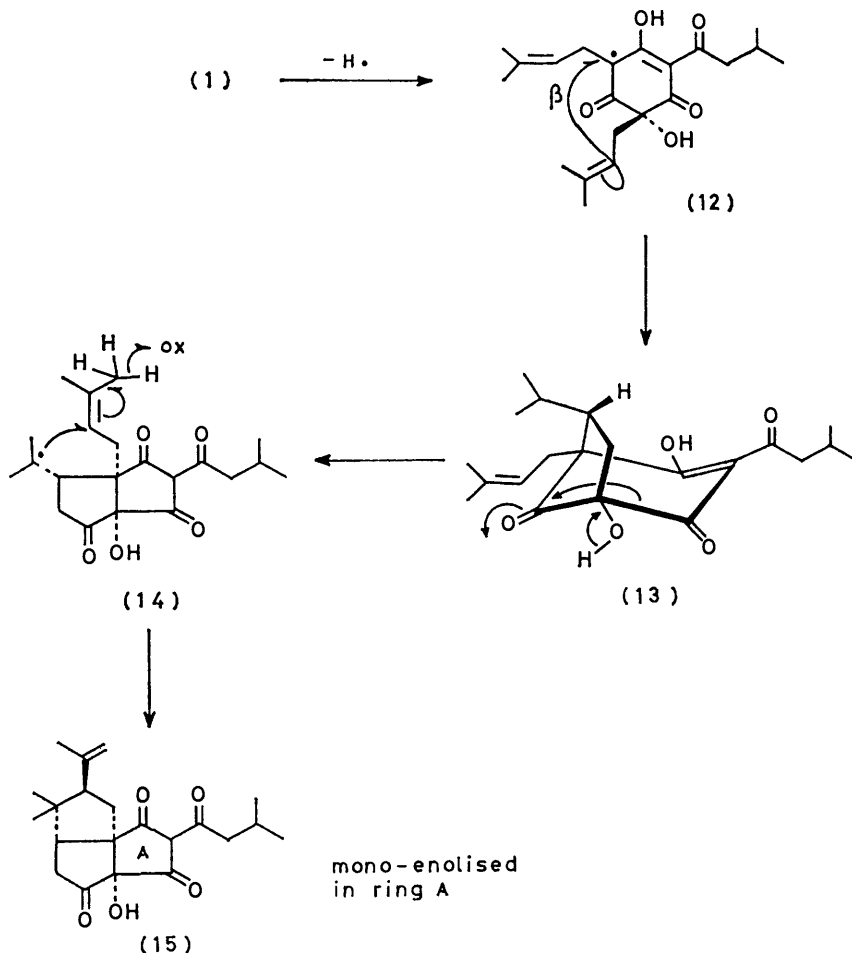
¹³C N.m.r. results for tricyclodehydroisohumulone (3)

Chemical shift δ (p.p.m.)	Intensity and multiplicity		Function	Assignment
208.1	m [a]	s [b]	C=O	6
205.9	m	s	C=O	In 3-substituent
199.1	m	s	C=O	4 (2)
191.3	w	s	C=O	2 (4), enolised
140.9	s	s	C=	In 10-substituent
112.4	s	t	CH₂=	In 10-substituent
108.5	w	s	C=	3 [c]
83.5	m	s	C—OH	5
57.5	m	s	—C̶—	1
56.7	s	d	CH	10
53.6	s	d	CH	8
46.0	m	t	CH₂	In 3-substituent
45.3	s	s	—C̶—	9
35.3	s	t	CH₂	7
30.7	s	t	CH₂	11
26.9	s	q	CH₃	In 10-substituent
25.2	s	q	CH	In 3-substituent
23.4	s	q	CH₃	At 9
22.2	s	q	2 × CH₃	In 3-substituent
16.2	s	q	CH₃	At 9

[a] From ¹H noise decoupled spectrum: s = strong, m = medium, w = weak. [b] From ¹H off-resonance decoupling: s = singlet, d = doublet, t = triplet, q = quartet. [c] Ref. 12.

(9)

(10)   (11)

only the latter in the ring-enolic (diosphenol) form (11) accommodated the strong i.r. absorption at 1 702 cm⁻¹, which was like that of 2-hydroxy-3-methylcyclopent-2-en-1-one,[14] and accounted for all of the features of the ¹H n.m.r. spectrum. As regards the methyl and methylene ¹H n.m.r. signals, these were similar to those in tricyclodehydroisohumulone, indicating an absence of skeletal rearrangement during the oxidation. The signal from the methylene group in the 3-methylbutanoyl side-chain was at a slightly higher field than that from the parent compound, in keeping with enolisation of the adjoining carbonyl group[8] in the newly formed β-dicarbonyl chain. The resulting adjacent vinylic proton, H_a in (11), gave a singlet at τ 4.48. Another distinctive

from an sp^2 hybridised carbon, evidently arose from the carbon atom at the centre of the β-tricarbonyl system, the chemical shift being similar to that found for the

feature comprised two one-proton doublets (J 3.3 Hz) at τ 3.44 and 6.68, either of which was collapsed by irradiation at the resonance frequency of the other: these signals were assigned respectively to H_b and H_c in (11). The magnitude of the coupling constant was typical for the geometry involved.[15]

With the assumption that no skeletal rearrangement was involved in the dehydrogenative coupling of the two side-chains derived from humulone, these various

merisation of humulone to isohumulone)[4,5] to yield the cis-fused bicyclic system (14) with the tertiary hydroxy-group and the 3-methylbut-2-enyl substituent both necessarily α-orientated. Oxidative cyclisation (to form ring c) would then complete the transformation. An examination of Dreiding molecular models confirmed the steric feasibility of the last three steps and suggested that the final ring-closure would lead to a β-orientation for the isopropenyl group. That direction of the final

observations thus led straightforwardly and unambiguously to the structure (3) for tricyclodehydroisohumulone. Suggestions concerning the stereochemistry of the molecule (3) can be made, taking the absolute configuration of humulone as shown in (1).[6,16]

For the primary act in the dehydrogenation of humulone (1) by lead tetra-acetate, formation of the radical (12) appeared most likely.[17] Further information came from the findings that humulone did not change or isomerise to isohumulone in the reaction medium in the absence of the oxidant and that attempted analogous oxidation of isohumulone (4) gave not even traces of tricyclodehydroisohumulone. As second step, electron-transfer cyclisation over the β-face of the radical (12) could occur to give (13). This intermediate radical might well undergo ring contraction (as in the iso-

ring-closure involves a methyl group–carbonyl group non-bonding interaction, which is preferable to the alternative methyl group–methylene interaction (involved in ring closure to give an α-isopropenyl group). Hence tricyclodehydroisohumulone may have the absolute configuration (15). However, the two steps of dehydrogenative ring closures might occur first with the ring contraction last. Examination of the Dreiding models for this alternative route shows again that rings A and B in the product would be cis-fused with an α-orientated tertiary hydroxy-group, but that the orientation of the isopropenyl group would be uncertain.

EXPERIMENTAL

with Perkin-Elmer 157G and Pye-Unicam SP 200 spectro-meters. ¹H n.m.r. (90 MHz) and ¹³C n.m.r. (22.6 MHz) spectra were obtained with respect to tetramethylsilane using a Bruker WH 90 Fourier-transform instrument. Mass spectra were recorded with A.E.I. MS 12 or Varian CH 5D instruments.

Tricyclodehydroisohumulone (3).—Solutions of humulone (6 g) and lead tetra-acetate (12 g) in glacial acetic acid were mixed (total volume, 250 ml) and rapidly cooled to 0 °C and the slurry stirred for 0.5 h. Water (500 ml) was added and the solution extracted with ether (2 × 500 ml). The extract was washed with water (2 × 500 ml), dried (MgSO$_4$), and evaporated, and the residue dissolved in acetic acid (12 ml). At 2 °C, crystals slowly separated: washing of these with acetic acid and drying (over KOH at ambient temper-ature and pressure) afforded an acetic acid *solvate* of (3) (2.4 g), m.p. 171 °C (decomp.), $[\alpha]_D^{20}$ *ca.* +250° (MeOH), *m/e* at 50 °C, 60 (acetic acid), and at 100 °C, 360 [compound (3)], $\nu_{max.}$(Nujol) 3 270 (OH), 1 760s (C=O), 1 715s (C=O), 1 675w (C=O), 1 630 and 1 570 (C=C), 1 375 and 1 150 (tert −OH, C−O), 1 250s (C−O, acetic acid), 990 and 925 cm⁻¹ (CH$_2$=C); ¹H n.m.r.—as for (3) plus τ(CDCl$_3$) 7.92 (3 H, s, Me of acetic acid; greatly diminished after solution had been shaken with D$_2$O). The solvate was unaffected in methanol with hydrogen sulphide (absence of Pb), and on t.l.c. on Kieselgel H was eluted with benzene–dimethyl ether (16 : 1 v/v) as one substance (R_F 0.6; u.v. light), visualised as a single orange spot by spraying with iron(III) chloride (1% in MeOH).

The acetic acid solvate (50 mg) was heated gradually in a thermogravimetric balance (Stanton): from 60 to 100 °C there was a smooth loss in weight (Found: loss, 14.4. C$_{21}$H$_{28}$O$_5$·C$_2$H$_4$O$_2$ requires 14.3%).

The acetic acid of crystallisation was removed (a) by shaking the solvate (1.2 g) with water (100 ml) for 18 h and (b) by sublimation at 150 °C and 2 × 10⁻⁴ mmHg. Re-crystallisation from light petroleum (b.p. 40—60 °C) afforded 5-*hydroxy-9,9-dimethyl-3-(3-methylbutanoyl)-10-(1-methylethenyl)tricyclo*[6.3.0¹,⁵.0¹,⁸]*undeca-2,4,6-trione* (tri-cyclodehydroisohumulone) (3) (overall yield 30%), m.p. 163 °C, $[\alpha]_D^{20}$ +299° (MeOH) (Found: C, 70.1; H, 7.7. C$_{21}$H$_{28}$O$_5$ requires C, 70.0; H, 7.8%), *m/e* 360.1949 (38%, M^+; calc. 360.1933) and 263.1283 (100%, B^+; calc. for C$_{15}$H$_{19}$O$_4$, 263.1283), $\lambda_{max.}$(acidic EtOH) [18] 237 and 268 nm (ε 10 500 and 11 000); $\lambda_{max.}$(alkaline EtOH) [18] 257 nm (ε 18 000); $\nu_{max.}$(Nujol) 3 380 (OH), 1 760s (C=O), 1 700s (C=O), 1 640s (bonded C=O), 1 565s (C=C), 1 380s, 1 350w, 1 325w, 1 305w, 1 290w, 1 265w, 1 220w, 1 205w, 1 170, 1 150, 1 105, 1 070w, 1 050, 990w, 925, 905s (C=CH$_2$), and 720 cm⁻¹; τ(CDCl$_3$) 9.46 (3 H, s, Me), 9.03 (6 H, d, *J* 6 Hz, *Me*$_2$CH), 8.93 (3 H, s, Me), 8.22 (3 H, s, MeC=C), 8.14—7.34 (9 H, c), 7.22 (2 H, *ca.* d, CH$_2$·CO), and 5.23br and 5.04br (each 1 H, CH$_2$=C).

Similar oxidation of humulone with lead tetra-acetate in propionic acid afforded the propionic acid *solvate* of (3), m.p. 208 °C (decomp.) (dried over KOH at ambient temper-ature and pressure), with a closely similar ¹H n.m.r. spec-trum to that of (3) plus signals at τ(CDCl$_3$) 8.84 (3 H, t, *J* 7 Hz, Me of propionic acid), and 7.62 (q, CH$_2$ of propionic acid).

Hydrogenation of Tricyclodehydroisohumulone.—The com-pound (3) (1 g) in methanol (200 ml) was shaken with Adams' catalyst (50 mg) under hydrogen until the uptake (187 ml at s.t.p.; 3 molar proportions) ceased (12 h). Removal of catalyst, evaporation of the filtrate under re-duced pressure, and crystallisation of the residue from

hexane afforded 5-*hydroxy-3-isopentyl-10-isopropyl-9,9-dimethyltricyclo*[6.3.0¹,⁵.0¹,⁸]*undeca-2,4,6-trione* (0.85 g, 85%), decomp. at 230 °C (Found: C, 72.1; H, 9.1%; *m/e* 348. C$_{21}$H$_{32}$O$_4$ requires C, 72.4; H, 9.3%, *M*, 348); $\lambda_{max.}$(acidic EtOH) [18] 259 nm (ε 8 500); $\lambda_{max.}$(alkaline EtOH) [18] 278 nm (ε 9 300); $\nu_{max.}$(Nujol) 3 370 (OH), 1 760s (C=O), 1 675w (CO), 1 410w, 1 310, 1 260w, 1 200w, 1 160w, 1 150w, and 1 100 cm⁻¹; τ[(CD$_3$)$_2$SO] 9.50 (3 H, s, Me), 9.16 (6 H, d, *J* 6 Hz, *Me*$_2$CH), 9.07 (6 H, d, *J* 6 Hz, *Me*$_2$CH), and 8.85—7.65 (17 H, c).

Periodate Oxidation of Tricyclodehydroisohumulone.[19]—Compound (3) (200 mg), dissolved in dioxan (8 ml), was treated with sodium metaperiodate (960 mg) in water (8 ml) at 20 °C for 2 weeks. After addition of water (*ca.* 15 ml) and extraction with diethyl ether (3 × 10 ml), the aqueous layer was acidified (HCl) and re-extracted with ether (2 × 10 ml). All the extracts were combined, dried (MgSO$_4$), and evaporated and the residue (124 mg) was chromatographed in ethyl acetate on silicic acid (Mallinck-rodt, 100 mesh). Gradient elution [ethyl acetate–light petroleum (b.p. 40—60 °C), latter increasing to 100%] gave finally the compound (11), 3-*hydroxy-1-(3-hydroxy-5-methylhex-2-enoyl)-6,6-dimethyl-7-(1-methylethenyl)bicyclo*-[3.3.0]*oct-3-en-2-one* (10.6 mg), isolated as an oil by evaporation [Found, field desorption *m/e* (%), 332 (100); M^+ (electron impact) 332.1986. C$_{20}$H$_{28}$O$_4$ requires *M*, 332.1987)], $\lambda_{max.}$(MeOH), 277 nm (ε 14 400); $\lambda_{max.}$(alkaline MeOH) [18] 297 nm (ε 18 000); $\nu_{max.}$(CHCl$_3$) 3 525 (OH) and 1 702 cm⁻¹ [=C(OH)·CO]; τ(CDCl$_3$) 9.43 (3 H, s, Me), 9.09 (6 H, d, *J* 7 Hz, *Me*$_2$CH), 8.82 (3 H, s, Me), 8.31 (3 H, s, MeC=C), 8.14—7.36 [6 H, c, which includes *ca.* 7.52, 2 H, *ca.* d, CH$_2$·C(OH)=], 6.66 and 3.44 (each 1 H, d, *J* 3.3 Hz, H$_c$, H$_b$), 5.43br and 5.17br (each 1 H, CH$_2$=C), 4.48 (1 H, s, H$_a$), 5.0—4.0 (1 H, OH), and −5.1 (1 H, chelated enol); *m/e* 332, 247, 205, 127, 85, 57, and *m/e* 247.1336 (*M* − C$_5$H$_8$O requires 247.1334).

REFERENCES

[1] Part 40, J. Coates, D. R. J. Laws, and J. A. Elvidge, *J.C.S. Perkin I*, 1974, 36.

[2] A. L. Whitear, *J. Inst. Brewing*, 1966, **72**, 177.

[3] J. A. Elvidge, D. R. J. Laws, J. D. McGuinness, and P. V. R. Shannon, *Chem. and Ind.*, 1974, 573, 671.

[4] J. S. Burton, R. Stevens, and J. A. Elvidge, *J. Inst. Brewing*, 1964, **70**, 345.

[5] F. Alderweireldt, M. Verzele, M. Anteunis, and J. Dierckens, *Bull. Soc. chim. belges*, 1965, **74**, 29; D. R. J. Laws and J. A. Elvidge, *J. Chem. Soc.* (C), 1971, 2412.

[6] D. De Keukeleire and M. Verzele, *Tetrahedron*, 1970, **26**, 385.

[7] M. Collins, D. R. J. Laws, J. D. McGuinness, and J. A. El-vidge, *J. Chem. Soc.* (C), 1971, 3814; B. E. Connett and J. A. Elvidge, *ibid.*, 1969, 340.

[8] J. S. Burton, J. A. Elvidge, and R. Stevens, *J. Chem. Soc.*, 1964, 3816.

[9] J. S. Burton, J. A. Elvidge, and R. Stevens, *J. Chem. Soc.*, 1965, 1276.

[10] P. M. Brown, G. A. Howard, and A. R. Tatchell, *J. Chem. Soc.*, 1959, 545.

[11] A. D. Cross and R. A. Jones, 'Introduction to Infrared Spectroscopy,' Butterworths, London, 1969, p. 85; K. E. Wiberg and B. J. Nist, *J. Amer. Chem. Soc.*, 1961, **83**, 1226; M. Nakazaki, K. Naemura, and S. Harita, *Bull. Chem. Soc. Japan*, 1975, **48**, 1907.

[12] F. Borremans, M. De Potter, and D. De Keukeleire, *Org. Magnetic Resonance*, 1975, **7**, 415.

[13] Sadtler Standard Spectra, no. 1615UV; A. I. Scott, 'Interpretation of the Ultraviolet Spectra of Natural Products,' Pergamon, Oxford, 1964, p. 69; E. M. Larsen, G. Terry, and J. Leddy, *J. Amer. Chem. Soc.*, 1953, **75**, 5107.

[14] Sadtler Standard Spectra, no. 19935.

[15] D. H. Williams and I. Fleming, 'Spectroscopic Methods in Organic Chemistry,' McGraw-Hill, New York, 1966, p. 124.

[16] D. De Keukelaire and M. Verzele, *J. Inst. Brewing*, 1970, **76**, 265.

[17] *cf.* R. Stevens and D. Wright, *J. Chem. Soc.*, 1963, 1763; P. R. Ashurst, P. M. Brown, J. A. Elvidge, and R. Stevens, *ibid.*, 1965, 6543.

[18] J. A. Elvidge and D. R. J. Laws, *J. Chem. Soc.* (*C*), 1968, 1193.

[19] A.-M. Davis, Ph.D. Thesis, Cardiff, 1977.