Structure and Absolute Stereochemistry of Pinguisone

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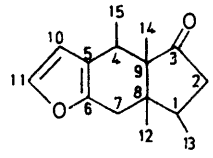
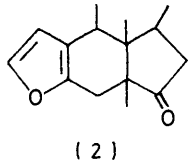
Structure (1) (4β,4aβ,7β,7aβ-tetramethyl-4,4a,6,7,7a,8-hexahydrocyclopenta[*f*]benzofuran-5-one) is definitively assigned to pinguisone on the basis of its mass and ^{13}C n.m.r. spectra; *X*-ray analysis of its *p*-bromobenzylidene derivative (4) establishes the absolute stereochemistry of the compound. The simultaneous formation of both (*Z*)- and (*E*)-*p*-bromobenzylidene derivatives (3) and (4) from (1) is discussed.

PINGUISONE is a crystalline compound with empirical formula $C_{15}H_{20}O_2$, isolated from the essential oil of *Aneura pinguis* (L.) Dum., a liverwort belonging to the genus Hepaticae.[1]

The structure of pinguisone (with no stereochemical implications) was formulated as (1) or (2) mainly through an analysis of its ^1H n.m.r. spectrum and a study of some simple derivatives.[2] Structures (1) and (2) have



(1)



(2)

a C_{15} skeleton and can be formally regarded as sesquiterpenoids,[2,3] although direct correlation with known members of this class of natural compounds is difficult. Similarly, their biosynthesis is hard to rationalize in terms of the isoprene rule, unless very unlikely molecular rearrangements are invoked.

The presence of four methyl groups bound to four adjacent carbon atoms is a notable feature of the structure of pinguisone, as is the lack of a β-methyl group in the furan ring, which is found in the most common furanoid sesquiterpenoids.[4]

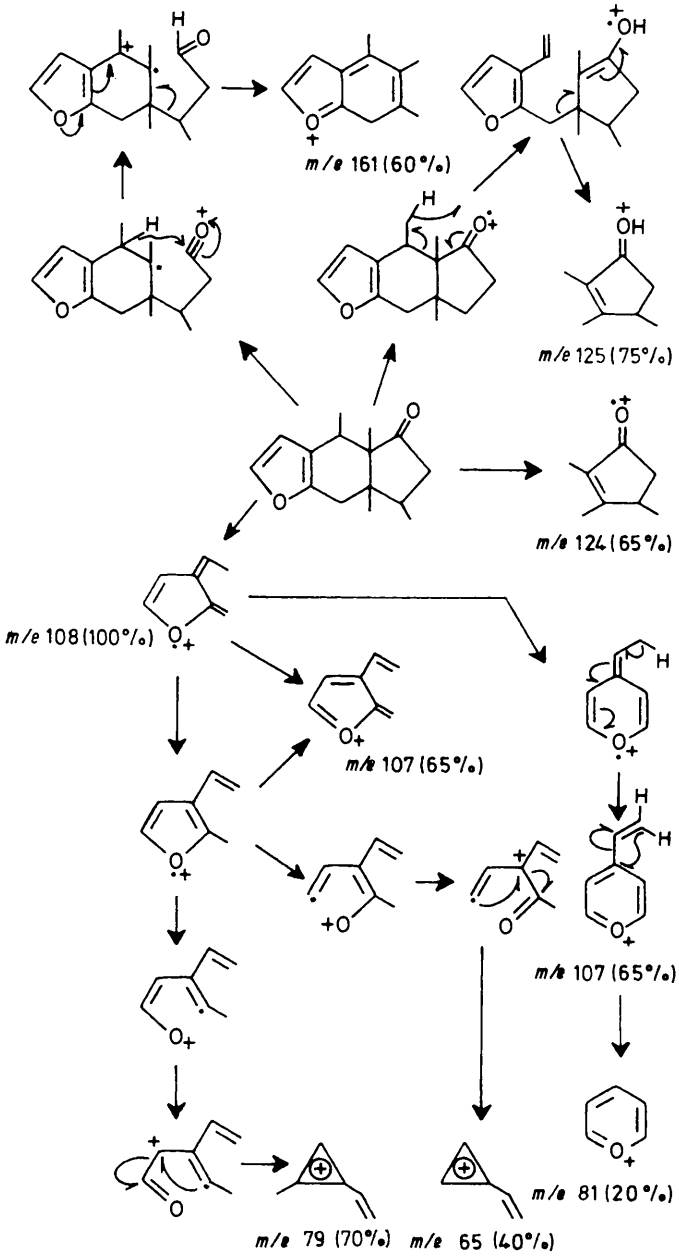
In connection with our work on the biosynthesis of sesquiterpenoids with unusual skeletons,[5] we are interested in the mechanism of formation *in vivo* of the pinguisane skeleton,[3,*] and this paper describes the

* We are grateful to Professor V. Herout and Dr. V. Benešová, who initiated our interest in this problem, helped us in collecting *A. pinguis* for biogenetic experiments, and made available a generous sample of pinguisone.

[1] V. Benešová, V. Herout, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 1810.
[2] V. Benešová, Z. Samek, V. Herout, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1969, **34**, 582.
[3] V. Herout, in 'Aspects of Terpenoid Chemistry and Biochemistry,' ed. T. W. Goodwin, Academic Press, London and New York, 1971, p. 68.
[4] T. K. Devon, and A. I. Scott, 'Handbook of Naturally Occurring Compounds, Vol. II, Terpenes,' Academic Press, London and New York, 1972, sect. 41.
[5] *E.g.* A. Corbella, P. Gariboldi, and G. Jommi, *J.C.S. Chem. Comm.*, 1972, 600; 1973, 729.

elucidation of the structure of pinguisone and the determination of its relative and absolute stereochemistry.

The mass spectrum of pinguisone exhibits a fragmentation pattern in accord with the structure (1). The most significant fragments are reported in the Scheme, where



SCHEME

the assigned structures are supported by high resolution spectra of (1) and of its [2-^{2}H]-derivative.

The presence of two oxygen atoms in (1) allows the formation of two radical cations which follow independent paths of fragmentation. The base-peak at *m/e* 108 originates from retro-Diels–Alder fission of the molecule, in agreement with the behaviour displayed by analogous furo-condensed cyclohexanes.[6] The other main fragments in the Scheme compare closely with those found in the mass spectra of other cycloalkanones [7] and of furanoid sesquiterpenoids.[8]

In view of the possible use of ^{13}C-labelled precursors in the study of the biosynthesis of pinguisone, we have recorded and fully assigned the ^{13}C n.m.r. spectrum of the compound (see the Table).

^{13}C Chemical shifts for pinguisone (0·5M in benzene) at 22·628 MHz; δ values in p.p.m. downfield from tetramethylsilane

C-1	29·51	C-6	147·99	C-11	141·17
C-2	41·53	C-7	28·53	C-12	18·91
C-3	215·26	C-8	45·50	C-13	13·84
C-4	32·11	C-9	56·48	C-14	8·84
C-5	117·77	C-10	109·26	C-15	13·52

The signal of the carbonyl C atom (C-3) [numbering as shown in (1)] which appears at a very low field, and the signals of the furan C atoms (C-5, C-6, C-10, and C-11) were assigned on the basis of chemical shifts and their off-resonance multiplicity.

The off-resonance quartets of the methyl carbons C-13 and C-15 and the doublets of the methine carbons C-1 and C-4 were attributed by single frequency decoupling of the corresponding protons whose assignments had previously been reported.[2,*]

The two angular methyl carbons C-12 and C-14 were differentiated on the basis of their multiplicity observed in the ^{13}C undecoupled spectrum: the C-14 quartet shows a hyperfine doublet splitting of 4·4 Hz due to the coupling with the proton at C-4, whereas each component of the C-12 quartet is a complex multiplet because of the coupling of the protons at C-7 and C-1. Although the coupling pattern of C-14 and C-12 does not rule out structure (2) for pinguisone, it largely favours structure (1) which accounts for the hyperfine splittings.

The off-resonance singlets of the quaternary carbons C-8 and C-9 were also assigned on the basis of their different multiplicity in the undecoupled spectrum and making allowance for the strong deshielding α-effect of the carbonyl group, relative to the β-effect. Finally, the two off-resonance triplets of the methylene carbons C-2 and C-7 were unequivocally attributed by comparison of the ^{1}H decoupled ^{13}C spectrum of pinguisone and of its dideuterio-derivative, in which the C-2 signal appears very broad due to coupling with deuterium.

Examination of the single frequency ^{1}H decoupled

* The ^{1}H spectrum quoted in the literature [2] was run in $CDCl_3$; a new proton spectrum of pinguisone was recorded in C_6D_6 at 270 MHz to get a more accurate set of values in the same solvent used for the ^{13}C spectrum.

[6] *E.g.* H. Budzikiewicz, C. Djerassi, and D. H. Williams, 'Structure Elucidation of Natural Products by Mass Spectrometry,' Holden-Day, San Francisco, 1964, pp. 104—105.

[7] See ref. 6, p. 65.

[8] N. Hayashi, S. Hayashi, and T. Matsuura, *Tetrahedron Letters*, 1968, 4957; N. Hayashi, *J. Sci. Hiroshima Univ.*, 1969, A-II **33**, 107.

signals of C-12 and C-14 in the ^{13}C spectrum, allowed identification of the corresponding resonances in the proton spectrum which had not been previously assigned;[2] these experiments show that the C-12 protons resonate at higher field (δ 0·46) than the C-14 protons (δ 0·72).

The carbonyl group of pinguisone must be very hindered from both sides of the molecule: it does not form any derivatives and cannot be reduced by $LiAlH_4$ under conditions compatible with the low stability of the compound. On the other hand, the methylene group α to the carbonyl displays normal reactivity, giving condensation products with aldehydes.[2]

In order to synthesize a derivative containing a heavy atom and suitable for *X*-ray analysis, pinguisone was treated with *p*-bromobenzaldehyde under basic catalysis. This condensation, even if run in the dark, afforded both the (*Z*)- and (*E*)-*p*-bromobenzylidene derivatives, which could be cleanly separated by rapid chromatography.

The Claisen–Schmidt condensation is known to afford the thermodynamically more stable isomer, which, for simple cyclic ketones [9] corresponds to the *trans* (*E*)-isomer; this isomer is also known to undergo photoisomerization to the *cis* (*Z*)-isomer.[10]

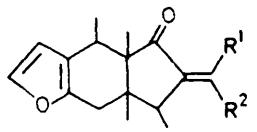
The direct simultaneous formation of both isomers (3) and (4) in this case can be rationalized in terms of a low energy difference between the transition states leading to the (*E*)- and (*Z*)-isomers: inspection of molecular models clearly shows that the steric hindrance induced by C-13 on the C-2 substituent is stronger in the more stable (*E*)-isomer than in the (*Z*)-isomer, thus accounting for an increase of the energy content of the former.

The more abundant isomer obtained from the condensation between pinguisone and *p*-bromobenzaldehyde is a white crystalline substance, m.p. 150—151°, $\lambda_{max.}$ 226 (ε 16,250) and 291 nm (21,000), $\nu_{max.}$ 1720, 1640, and 750 cm^{-1}. In the ^1H n.m.r. spectrum the most significant signal is at δ 7·42 attributable to the vinyl proton of the benzylidene group.

The minor component forms brilliant yellow crystals, m.p. 156—157°, $\lambda_{max.}$ 222 (ε 15,500) and 291 nm (21,900). In the ^1H n.m.r. spectrum the vinyl proton resonates at δ 6·45, and the i.r. spectrum shows bands at 1717, 1645, 1625, and 730 cm^{-1}.

Cromwell [10] has pointed out that for αβ-unsaturated exocyclic ketones, only ^1H n.m.r. is discriminating when the configuration of the double bond has to be ascertained. In our case, the vinyl proton in the more abundant isomer resonates at lower field (*ca.* 1 p.p.m.) than the other isomer; such a deshielding effect, due to the anisotropy of the carbonyl group, allows assignment of an (*E*)-configuration (3) to the former and a (*Z*)-configuration (4) to the latter.

Considering the other properties of (3) and (4), the slightly higher m.p. of the (*E*)-isomer (3) is in contrast with the normal expectation; [11] furthermore, the u.v. spectra are very similar, thus indicating that steric hindrance in both configurations causes incomplete overlapping of the π-orbitals of the conjugated system.



(3) R^1=H, R^2=C_6H_4Br-*p*

(4) R^1=C_6H_4Br-*p*, R^2=H

U.v. irradiation of (3) in different solvents affords a photostationary mixture containing 75% of (3) and 25% of (4). Also, (3) and (4) undergo chemical equilibration under acidic or basic catalysis, or after prolonged contact with silica gel; the composition of the equilibrium mixture is always very near to that reported above for photoisomerization.

The *p*-bromobenzylidene derivative (4), but not (3), formed crystals suitable for *X*-ray analysis, which definitely confirmed the structure of pinguisone and indicated its absolute stereochemistry.

X-Ray single crystal analysis afforded for (4) the following data: $C_{22}H_{23}BrO_2$, $M = 399·3$. Orthorhombic, $a = 15·252(10)$, $b = 11·728(3)$, $c = 10·586(3)$ Å, $U = 1893·6$ Å^3, $D_m = 1·4$, $Z = 4$, $D_c = 1·40$ g cm^{-3}, $F(000) = 824$. Space group $P2_12_12_1$. Cu-K_α radiation μ(Cu-K_α) = 33·4 cm^{-1}, $\lambda = 1·5418$ Å.

Integrated intensities were measured on a Picker single-crystal diffractometer for *hkl* and *hkl* reflections having $\sin\theta/\lambda < 0·56$ Å^{-1} and 1753 were considered observable (1072 with $h \geqslant 0$, $k \geqslant 0$, $l \geqslant 0$ and 681 with $h > 0$, $k > 0$, $l < 0$). No absorption correction was introduced ($\mu R \simeq 0·8$).

The structure was elucidated by the heavy atom method, the bromine atom being located from a three-dimensional Patterson synthesis and the light non-hydrogen atoms by two successive Fourier syntheses. Anisotropic least-squares refinement was performed twice on the two enantiomeric structures, taking into account the anomalous scattering for the bromine atoms. The *R* factor was reduced to 0·055 for the configuration shown in the Figure (in the absolute sense) and to 0·065 for the opposite one. Moreover we have considered the 37 reflections having calculated structure factors for the two enantiomers F_{calc}^+ and F_{calc}^- differing by at least 1·5 electrons. For 36 out of 37 reflections the difference $F_{calc}^+ - F_{calc}^-$ has the same sign as $F_{obs}(hkl) - F_{obs}(\bar{h}\bar{k}\bar{l})$. The absolute configuration shown in the Figure is thus proved.

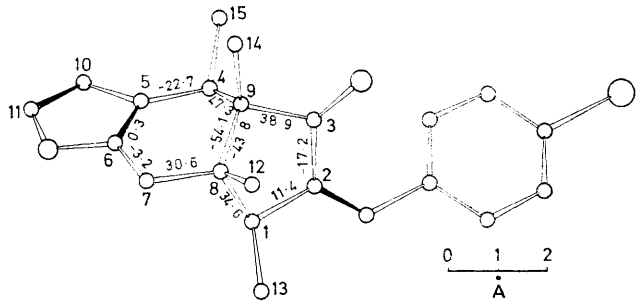
The junction between the five- and six-membered rings is *cis* and the four methyl groups are also mutually *cis*. Both rings exhibit rather unusual conformations and torsion angles are indicated in the Figure. In the

[9] H. O. House, 'Modern Synthetic Reactions,' W. A. Benjamin, Menlo Park, California, 1972, pp. 635—636.

[10] D. N. Kevill, E. D. Weiler, and N. H. Cromwell, *J. Org. Chem.*, 1964, **29**, 1276.

[11] E. L. Eliel, 'Stereochemistry of Carbon Compounds,' McGraw-Hill, New York, 1962, pp. 326—327.

six-membered ring, five of the six atoms lie approximately in a plane (the cyclohexene itself has the half-chair conformation [12] with torsion angles 45, −15, 0, −15, 45, and −62°). In the five-membered ring, on the other hand, a puckered arrangement close to the envelope conformation [13] is present. These conformations are clearly induced by the *cis* junction of the two



Molecular model of the (*Z*)-*p*-bromobenzylidene derivative (4) of pinguisone, in its absolute configuration. The double bonds are drawn solid. The torsion angles (deg.) for the five- and six-membered rings are indicated.

rings. The torsion angles for the couples of vicinal methyl groups are: 43·8° for C-14 and C-15, −48·7° for C-14 and C-12, and 42·3° for C-12 and C-13.

Considerable distortions of the valence angles are present in the cyclopentanone ring: the angles at C-1, C-2, C-3, C-9, and C-8 are respectively 103·6, 107·0, 108·0, 99·9, and 101·1°. The valence angles at C-4, C-5, C-6, C-7, C-8, and C-9 in the six-membered ring are 108·6, 123·5, 128·6, 110·3, 112·6, and 111·3°. Only the value 128·6° appreciably deviates from the cyclohexene angles.[12] No significant distortions are present in bond lengths.

Preliminary experiments on the biosynthesis of pinguisone have been hampered by the insignificant incorporation yields of labelled precursors. The total synthesis of pinguisone is under investigation.

EXPERIMENTAL

M.p.s were measured on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. U.v. spectra were recorded in iso-octane solution on a Beckmann DB-GT grating spectrophotometer, i.r. spectra on a Perkin-Elmer 257 Infracord spectrophotometer, mass spectra on an AEI MS902S instrument, and ^{1}H n.m.r. on a Bruker HFX 90 and 270 spectrometer. ^{13}C N.m.r. spectra were recorded on the latter instrument equipped with a P.F.T. Nicolet 1080 system.

Preparation of [2-^{2}H]*Pinguisone*.—Pinguisone (100 mg) was dissolved in MeOD and treated with a catalytic amount of sublimed potassium t-butoxide. After 1 h at room temperature D_2O (0·5 ml) was added and the solution concentrated *in vacuo*. The organic material was extracted three times with carbon tetrachloride and the solvent evaporated. The same procedure was repeated three times on the same material thus obtaining almost complete deuteriation, as shown by mass spectral analysis.

Preparation of the p-*Bromobenzylidene Derivatives* (3) *and* (4).—Pinguisone (100 mg) in anhydrous methanol (5 ml) was treated with *p*-bromobenzaldehyde (75 mg) and potassium t-butoxide (80 mg). The solution was refluxed 14 h, then concentrated under reduced pressure. The residue was partitioned between water and ether and the aqueous layer extracted three times with the same solvent. Evaporation of the organic extracts yielded a residue (150 mg) which gave two new spots in t.l.c. near some unchanged material. The two compounds were separated by preparative t.l.c. on three 20 × 20 silica-gel plates (Merck F 254; 0·25 mm) after three successive elutions with benzene.

The less polar (*Z*)-isomer (4) (R_F 0·6) crystallized from n-hexane in brilliant yellow prisms (12 mg), m.p. 156—157°, $[\alpha]_D^{20}$ +417° (*c* 0·3 in CCl_4), $\lambda_{max.}$ 222 (ε 15,500) and 291 nm (21,900), $\nu_{max.}$ (Nujol) 1717, 1645, 1625, and 730 cm^{-1}, δ (CCl_4) 0·84 (6H, s, 12- and 14-CH_3), 1·07 (3H, d, *J* 7 Hz, 13- or 15-CH_3), 1·15 (3H, d, *J* 6·8 Hz, 15- or 13-CH_3), 6·11 (1H, d, *J* 1·7 Hz, H-10), 6·45 (1H, d, *J* 2·7 Hz, =C*H*·Ar), 7·17 (1H, m, H-11), and 7·41 and 7·69 (4H, 2d, *J* 8·5 Hz, ArH).

The more polar (*E*)-isomer (3) (R_F 0·5) crystallized from n-hexane in white fibres (80 mg), m.p. 150—151°, $[\alpha]_D^{20}$ +277° (*c* 1 in $CHCl_3$), $\lambda_{max.}$ 226 (ε 16,250) and 291 nm (21,000), $\nu_{max.}$ (Nujol) 1720, 1640, and 750 cm^{-1}, δ (CCl_4) 0·82 (3H, s, 12- or 14-CH_3), 0·88 (3H, s, 14- or 12-CH_3), 0·95 (3H, d, *J* 6·8 Hz, 13- or 15-CH_3), 1·07 (3H, d, *J* 7·0 Hz, 15- or 13-CH_3), 6·16 (1H, d, *J* 1·7 Hz, H-10), 7·20 (1H, m, H-11), 7·42 (1H, m, =C*H*·Ar), and 7·19 and 7·41 (4H, 2d, *J* 8·5 Hz, ArH).

Unchanged pinguisone (25 mg) was recovered from the t.l.c. plates (R_F 0·3).

Chemical Equilibration of (3) *and* (4).—The (*E*)-isomer (3) (3 mg) was dissolved in a 0·1M solution of piperidine in acetonitrile (1 ml). After 5 h at room temperature complete equilibration occurred yielding a mixture of (3) and (4) in a ratio of 4 : 1 as shown by ^{1}H n.m.r. analysis. The same treatment on the (*Z*)-isomer took 1 week at 70—80° to achieve complete equilibration.

Photoisomerization of (3).—The (*E*)-isomer (3) (73 mg) in carbon tetrachloride (10 ml) was irradiated under nitrogen in a Pyrex vessel with an HPK 125 W Philips u.v. lamp. After 20 min photoequilibration was complete and the yellow solution was evaporated. Preparative t.l.c. as described above gave the starting material (49 mg) and its (*Z*)-isomer (4) (12 mg). No appreciable differences were noted on changing the polarity of the solvent (methanol, benzene, and iso-octane were used).

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[12] F. Chiang, and S. H. Bauer, *J. Amer. Chem. Soc.*, 1969, **91**, 1898.

[13] E. L. Eliel, H. L. Allinger, S. J. Angyal, and G. A. Morrison, 'Conformational Analysis,' Interscience, New York, 1967, p. 200.