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THE STRUCTURE OF BRUCEOL

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Abstract—A novel coumarin, bruceol, has been isolated from *Eriostemon brucei* F. Muell. Preliminary chemical studies have been made and the complete structure determined by X-ray crystallography of a monobromo derivative.

INTRODUCTION

As PART of the survey[1,2] of members of the Rutaceae from arid regions of Western Australia a preliminary examination of *Eriostemon brucei* has been made. The plant is reported[3] to have been used by aborigines for alleviation of colds by inhalation from the heated leaves, and this might be explained by our observation of a high (~70%) proportion of cineole in the essential oil.

Partition of the ether extract gave an acid, $C_{19}H_{22}O_6$, a neutral optically active coumarin, $C_{19}H_{20}O_5$, which we have called bruceol, and the alkaloid, maculosidine. The latter was originally isolated from *Flindersia maculata*,[4] and is a common constituent of Eriostemon spp.[1,2] The presence of a coumarin system in bruceol is suggested by the U.V. spectrum measured for an ethanol solution (λ_{max} 218, 254, 260 and 330 with a shoulder at 235 mμ, log ε 4·43, 3·69, 3·69, 4·13 and 4·02 respectively), and by its solubility in hot sodium hydroxide solution, from which it can be recovered together with the *trans*-hydroxycinnamic acid. The latter is identical with the acid isolated directly from the plant material and, in view of the mild conditions when used in isolation, it is unlikely to be an artifact.

Bruceol contains a hydroxyl group which is readily characterized by the usual derivatives. Infrared absorption at 3582 cm^{-1} ($CHCl_3$) suggests the group is intramolecularly hydrogen bonded. Absorptions at 1721 cm^{-1} and 1623 cm^{-1} are assigned to the coumarin carbonyl and conjugated olefin respectively. Hydrogenation with a platinum catalyst gave a dihydro derivative which must be the 3,4-dihydrocoumarin since the U.V. absorption spectrum (λ_{max} 220 and 275 mμ; log ε 4·48 and 3·11) corresponds to an unconjugated benzene chromophore. In the infrared the carbonyl group has absorption at 1745 cm^{-1} but no absorption hear 1621 cm^{-1} could be detected. In addition dihydrobruceol is a monohydric alcohol and hence bruceol contains no other carbonyl groups.

Bruceol and a number of its derivatives showed I.R. absorption near 1380 and 1360 cm^{-1}, suggesting the presence of a *gem*- dimethyl group and this was substantiated

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[1] A.M. Duffield, P. R. Jefferies and P. H. Lucich, *Aust. J. Chem.* **15** (1962).
[2] A. M. Duffield and P. R. Jefferies, *Aust. J. Chem.* In the press.
[3] A. T. Monck (1946). File 2274/46. Dept. of Industrial Development, Western Australia.
[4] R. F. C. Brown, P. T. Gilham, G. K. Hughes and E. Ritchie, *Aust. J. Chem.* **7**, 181 (1954).

from chromic acid oxidation of bruceol which afforded acetone. No methoxyl or methylenedioxy groups could be detected and hence bruceol must contain two ether oxygen atoms. Assuming that the unsaturation in bruceol is entirely due to the coumarin system then bruceol must be pentacyclic and the identification of the C_6—C_3 unit suggests the residual carbon content is based on two isopentyl units.

At this stage of the investigation difficulty was found in securing further quantities of bruceol and, in the meantime, a monobromo derivative termed bromo-bruceol was prepared for crystallographic analysis by treating bruceol with a dioxane–bromine solution in the presence of sodium carbonate.

PRELIMINARY INVESTIGATION

Crystals suitable for an X ray diffraction study were obtained on cooling a solution of bromo-bruceol in hot benzene. The crystals were acicular in shape and showed extinction along the needle axis when examined under a polarizing microscope. A crystal was mounted along this axis and a series of oscillation and Weissenberg photographs were taken using CuKα X radiation. The cell dimensions, measured from these photographs, are:

$$a = 7{\cdot}26 \text{ Å}, \qquad b = 17{\cdot}9 \text{ Å}, \qquad c = 26{\cdot}8 \text{ Å}.$$

The a axis corresponds to the needle axis of the crystal. The reflection conditions were observed to be hkl, $h + k = 2n$; OOl, $l = 2n$ and these uniquely determine the space group as $C222_1$.

A crystal of approximate dimensions $0{\cdot}8 \times 0{\cdot}05 \times 0{\cdot}03$ mm^3 was mounted about the a axis. A series of equi-inclination Weissenberg photographs of the layers $h = 0$ to 6 were taken using CuKα radiation. Crystals of approximate dimensions $0{\cdot}1 \times 0{\cdot}1 \times 0{\cdot}03$ mm^3 were mounted about the b and c axes and Weissenberg photographs of the b axis layers $k = 0$ to 3 and the c axis zero layer were recorded. The intensities of the reflections were estimated by visual comparison with a standard scale, 1,141 of the 1,990 independent reflections in the CuKα sphere being recorded. The intensities were corrected for Lorentz, polarization and inclination factors, but no corrections were made for absorption.

On comparing the common row lines of the a axis and b axis films, significant differences in relative intensities were observed for some of the reflections. These were not systematic with Bragg angle and, consequently, could not be ascribed to differences in the thermal motions of the atoms in the two crystals. It therefore appeared that some change in the structure had taken place. Further photography showed that this change occurred with time, rather than X ray exposure, as photographs of crystals which had not previously been exposed to X-rays, but which were of different ages, did not correlate. The a axis intensity data was expected to have been only slightly affected by this degradation as the relevant photographs had all been recorded with fresh crystals. However, as the correlation with the b axis photographs, which had been taken with older crystals, was poor, only approximate layer-to-layer scaling factors could be determined. The data was reduced to an approximately common scale using these factors, which were subsequently corrected during the structure refinement.

LOCATION OF THE BROMINE ATOM

Patterson syntheses were evaluated for the projections down the (100) and (010) axes using data modified by the function $M(\theta) = \hat{f}^{-2} \exp(2B \sin^2\theta/\lambda^2)$ where θ is

the Bragg angle, $\hat{f}$ is the mean unitary scattering factor, B is the mean isotropic temperature factor, determined from a Wilson plot on the (*Okl*) data to be 2·38 Å and λ is the wavelength of CuKα radiation. The effects of this modification function on the Patterson function have recently been investigated.[5,6] In both projections peaks corresponding to the bromine-bromine vectors are considerably higher than any others. The coordinates determined from them for the bromine atom are $x/a = 0{\cdot}234$, $y/b = 0{\cdot}132$, $z/c = 0{\cdot}227$. The fractional x-coordinate of the bromine atom is very nearly equal to $\frac{1}{4}$. In fact, the position of the Patterson peak maxima implied that x was exactly equal to $\frac{1}{4}$ and the small displacement from this value was deduced from the fact that the peaks were extended in the x direction. Had the bromine atom been placed on the $x = \frac{1}{4}$ plane, the phases of its contribution to the structure factors would have been restricted to the values 0, $\pi/2$, π or $3\pi/2$ radians, and false mirror planes across the planes $x = \frac{1}{4}$ and $x = \frac{3}{4}$ would have appeared in Fourier syntheses based on these phases alone. Even with $x = 0{\cdot}234$, some false symmetry of this type was expected to occur.

TWO-DIMENSIONAL ANALYSIS

An electron density projection perpendicular to a, the shortest axis, was calculated with the terms phased on the bromine contribution. This gave no indication of the chemical configuration and atoms were placed on the twelve most significant peaks, without regard to stereochemistry. Structure factors were calculated which gave an R factor of 50%. Another electron density map and an error synthesis, calculated using the phases of these structure factors, led to the elimination of one of the original twelve and the postulating of a further six atoms. Structure factors on this trial structure gave an R-factor of 44%. This procedure was continued until, after eight rounds, 23 atomic sites had been selected and the R-factor had decreased to 31%, but the positions still gave little indication as to the stereochemistry of the molecule. At this stage the results of the three-dimensional analysis were becoming available and the two-dimensional work was discontinued. Comparison later with the correct structure showed that the majority of the positions postulated from the two-dimensional work were correct, but the chemistry was concealed by the overlapping of two molecules in this projection.

THREE-DIMENSIONAL ANALYSIS

(i) *Determination of the molecular configuration*

Phases based on the bromine atom were calculated for the complete set of data and a full three-dimensional electron density map was evaluated. The section at $x/a = \frac{8}{30}$ is shown in Fig. 1. The concentration of electron density in this section is high, and the form of the distribution is such that a double ring configuration of the form required for a coumarin system could be postulated. As this was supported by the chemical evidence, atoms 1 and 20 were assumed to be oxygens. (For the numbering of the molecule, see Fig. 2.) In addition to the coumarin system, there were peaks at the O_{11}, C_{12}, C_{15}, C_{16}, O_{17}, C_{21} and C_{23} positions, all of which were close to the $x = \frac{1}{4}$ plane, and, for the next structure factor calculation, carbon atoms were

[5] S. Abrahamsson and E. N. Maslen, *Acta Cryst.* **13**, 1001 (1960).

[6] S. Abrahamsson and E. N. Maslen, to be published.

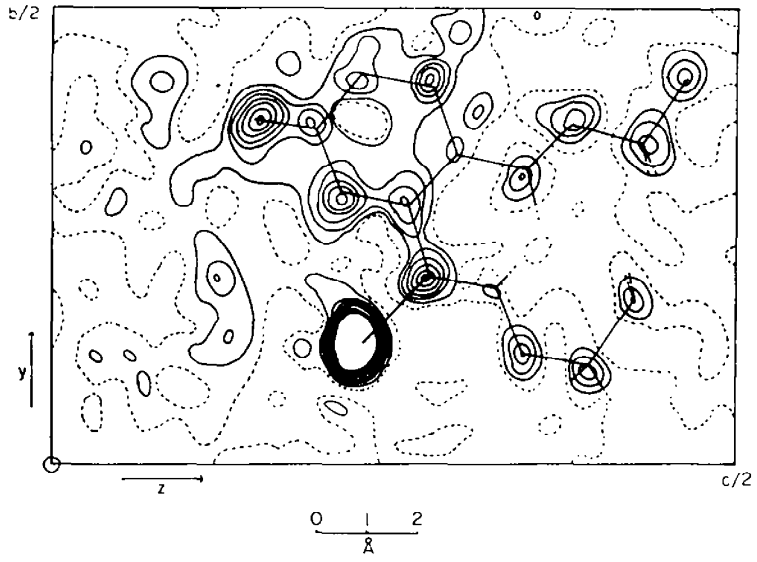


FIG. 1. The section through $x/a = 8/30$ of the bromine phased three-dimensional electron density map. First contour (broken) at $\frac{1}{2}$ eÅ^{-3}; contour interval 1 eÅ^{-3}; 65 contours omitted in the bromine peak. The section of the molecular skeleton lying between $x/a = 6/30$ and $10/30$ is shown.

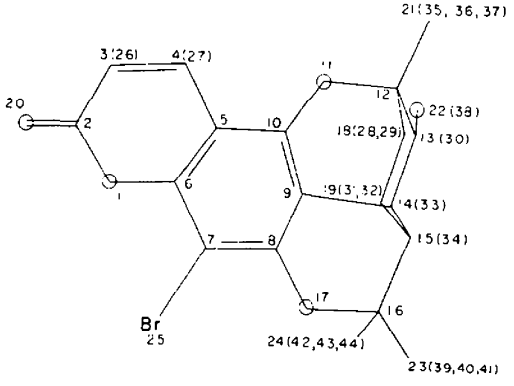


FIG. 2. The molecular formula of bromo-bruceol. The diagram is numbered in accordance with the numbering system used throughout the paper.

placed at these sites. Peaks also appeared at positions later found to correspond to the C_{13}, C_{14}, C_{18} and C_{19} atoms, but, as C_{13} and C_{14} are approximate mirror images across the $x = \frac{1}{4}$ plane of C_{18} and C_{19}, it was considered probable at that time that one pair was a pseudo-mirror of the other. In this space group, there is a degree of freedom associated with the direction of displacement from the $x = \frac{1}{4}$ plane. As all the other atoms lay on or near the plane, this allowed one of the four peaks to be arbitrarily assigned to an atom which, when included in the structure factor calculation, removes the false symmetry. The peak at C_{14} was selected for this purpose.

Structure factors were evaluated, based on the 20 atomic positions postulated from the first electron density map. The temperature factor of 2·38 Å, derived from

the (*Okl*) Wilson plot was used for the carbon and oxygen atoms and the bromine was given the value of 3·00 Å^2, obtained from the two-dimensional refinement. The scattering factors used were, for bromine, that of Thomas and Umeda,[7] corrected for anomalous dispersion using the parameters of Dauben;[8] for carbon, that of Berghuis *et al.*;[9] and for oxygen, that of Freeman.[10] The R-factor on this set of structure factors was 31%. Examination of the agreement showed that the layer-to-layer scaling was poor, as was expected in view of the difficulties encountered in the scaling process.

A second three-dimensional electron density map was calculated using the phases of these structure factors. All the atoms which had been included in the trial structure lay on peaks in the syntheses, but that associated with C_3 was about 1·5 eÅ^{-3} lower than the others and it therefore was suspected that this atom took part in the degradation of the molecule. Five peaks of about 3 eÅ^{-3} in height. which were not associated with included atoms, were observed. Among these were peaks corresponding to the C_{13}, C_{18} and C_{19} atoms, which had been observed in the first electron density map, but omitted from the structure factor calculation. Their persistence indicated that they were all real atomic peaks and the existence of the cyclohexane ring system was established. The remaining two peaks were associated with O_{22} and C_{24}, completing the molecular skeleton, except for hydrogens. However, only one more oxygen (O_{17}) lay on a peak sufficiently high to distinguish it from a carbon peak.

Structure factors were calculated for the 25 atomic positions postulated, atoms O_1, O_{17} and O_{20} being weighted as oxygens. The temperature factors were identical to those used in the previous round and the layer-to-layer scaling factors were adjusted using the appropriate $\Sigma|Fo|/\Sigma|Fc|$ ratios obtained from the first structure factor calculation. The R-factor on these structure factors was 20%. An electron density map and difference synthesis were evaluated using these structure factors. The structure as postulated was confirmed and the relative peak heights supported the identification of atoms O_1, O_{17} and O_{20} as oxygens. The chemical evidence suggested that the remaining two oxygens were at the 11 and 22 positions. The peak for the 22 atom in the electron density map was about 2 eÅ^{-3} higher than the average carbon peak and there was a corresponding peak of about 2 eÅ^{-3} in the difference map. The case for atom 11 being an oxygen was not so conclusive. The peak in the electron density map was no higher than those for some of the carbons; there was a peak of about 2·5 eÅ^{-3} in height in the difference synthesis, but it was some way from the postulated atomic positon. This indicated a shift in the atomic position, but in view of the considerable height of the peak and the strong support from the compound's chemical behavior, the atom was identified as oxygen. This identification was supported by the subsequent refinement.

(ii) *Location of the hydrogen atoms*

Approximate positions had now been determined for all the atoms in the structure, except for the hydrogens. An examination of the molecular bonding arrangement

[7] L. H. Thomas and T. Umeda, *J. Phys. Chem.* **26**, 293 (1957).
[8] C. H. Dauben, *Acta Cryst.* **8**, 478 (1955).
[9] J. Berghuis, I. M. Haanappel, M. Potters, B. O. Loopstra, C. H. MacGillavry and A. L. Veenendahl, *Acta Cryst.* **8**, 478 (1955).
[10] A. J. Freeman, *Acta Cryst.* **12**, 261 (1959).

shows that, of the nineteen hydrogen atoms, the positions of nine are sterically determined by the framework of the rest of the molecule and the remaining ten are contained in the three methyl and the hydroxyl groups. Assuming C—H bond lengths of 1·09 Å, the positions of these first nine hydrogens were evaluated. The positions corresponded in every case to positive regions in the difference synthesis and, in many cases, to actual peaks of slightly less than 1 eÅ^{-3} in height. Encouraged by this, a search was made for the remaining hydrogens, whose positions were not completely specified by the heavy atom positions. For the methyl groups, the planes expected to contain hydrogen atoms were plotted (Fig. 3). The three hydrogen atoms of a methyl group should lie 120° apart on the circumference of a circle of radius 1·03 Å, centred at the intersection of the produced C—C bond and the plane. As can be seen, the orientation of the group can be deduced in every case from the difference electron density sections. A similar examination of the difference map in the vicinity of the atom O_{22} gave a probable position for the hydroxyl hydrogen.

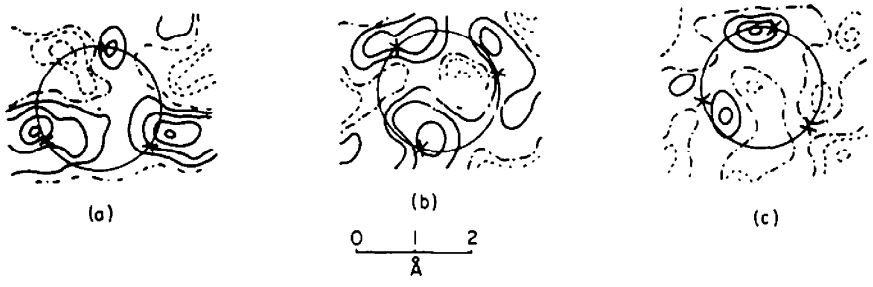


FIG. 3. Sections of the second difference synthesis through the planes containing the hydrogen atoms associated with C_{21} (a), C_{22} (b) and C_{24} (c). The hydrogen atoms should lie on the circumferences of the circles shown and their postulated positions are indicated. Zero contour "dot-dash" negative contours broken; contour interval ½ eÅ^{-3}.

Although the evidence for the hydrogen positions is consistent and encouraging, it is not conclusive as spurious peaks of up to 2 eÅ^{-3} occurred elsewhere in the difference map. Nevertheless, sufficient confidence was felt to include the contributions of all nineteen hydrogens to the structure factors in the subsequent refinement, although the hydrogen parameters were not themselves refined.

(iii) *Refinement of the structure*

In the second difference synthesis, the bromine atom lay on an elongated peak which was flanked by two hollows. This indicated that the mean temperature factor of the bromine was too high and that its thermal motion was anisotropic. The principal direction of vibration appeared to lie in the plane of the coumarin system, approximately perpendicular to the bromine-carbon bond direction. There was no appreciable evidence for anisotropy in the thermal motions of the light atoms, or for discrepancies in their isotropic temperature factors.

No adjustments were made to the temperature parameters on the basis of the difference synthesis, but both the positional and the thermal parameters of all the atoms except the hydrogens were refined by least squares methods using a programme written for the MERCURY computer by Dr. J. S. Rollet of the University of Oxford. The refinement with this programme, which uses a block diagonal approximation

to the full least squares matrix, was continued for six rounds at which stage the R-factor had been reduced to 15·6%.

Despite the lack of evidence in the first difference synthesis for changes in the light atom temperature factors, many of the thermal parameters obtained by least squares were very large and highly anisotropic. In fact, the thermal parameters of some of the atoms were as high as 20 Å^2. Electron density and difference maps were evaluated, but they gave no evidence for any lowering of these values. In view of this it was considered advisable to conduct an independent check for gross error

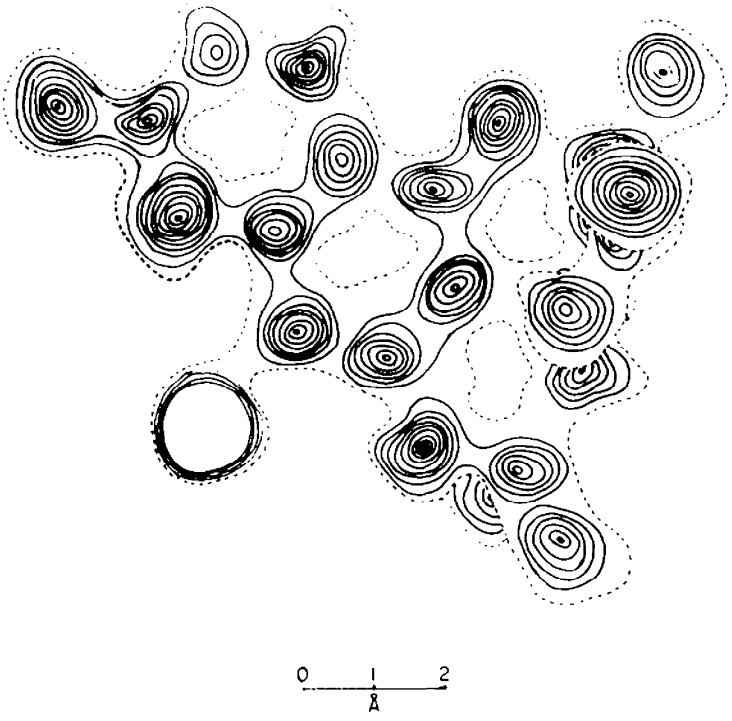


FIG. 4. The final electron density map, summarized down a, over the atomic centres. First contour (broken) at ½ eÅ^{-3}; contour interval 1 eÅ^{-3}; 60 contours omitted in the bromine peak.

in the positioning of these atoms. Accordingly, structure factors were calculated omitting atoms C_3, C_{16}, C_{19} and C_{21}, which exhibited the most serious temperature factor divergence, and an electron density map was evaluated using the phases of these structure factors. Significant peaks occurred in the vicinity of each omitted atom. It was concluded that the atoms were not grossly misplaced and the temperature factor divergence was attributed to the indirect action of the inverse overlap effects discussed below.

A summary of the final electron density map, taken parallel to a over the atomic centres is given in Fig. 4, and 5 shows a similar summary parallel to c over the cyclohexane system, the three methyl groups and the hydroxyl. The section $x = 8a/30$ of the final electron density map is given in Fig. 6 which should be compared with Fig. 1, which shows the same section of the bromine-phased map.

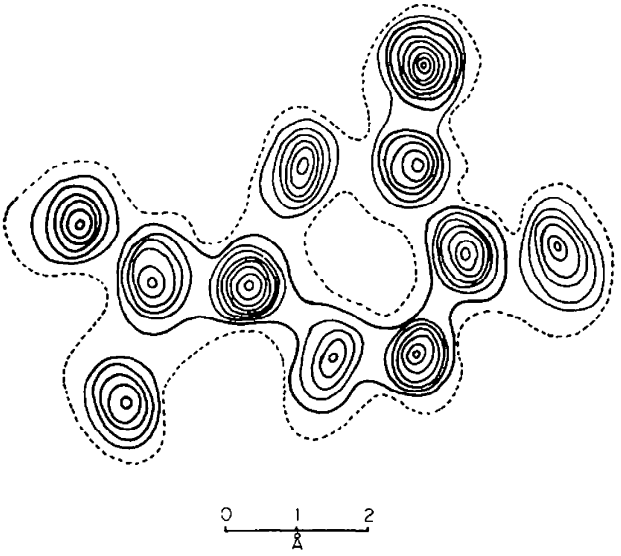


FIG. 5. The final Fourier synthesis, summarized down c, over the cyclo-hexane ring and the three methyl groups. First contour (broken) at ½ eÅ^{-3}; contour interval 1 eÅ^{-3}.

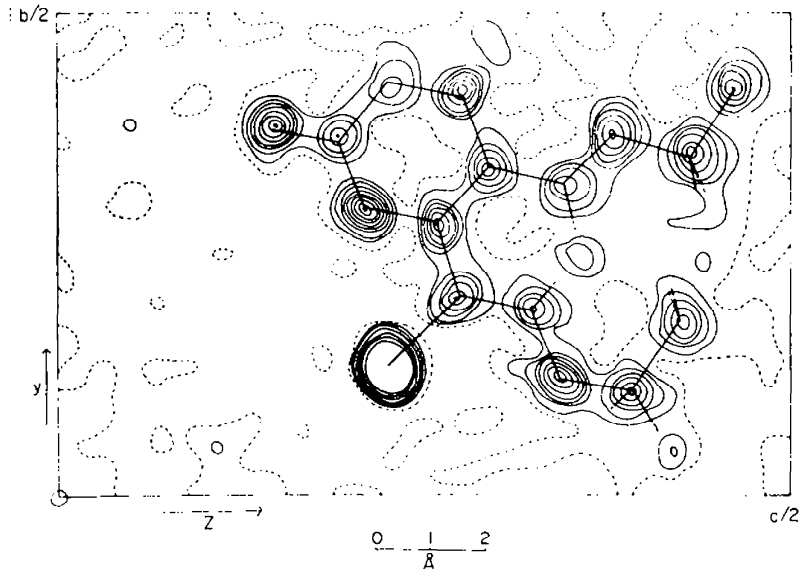


FIG. 6. The section $x/a = 8/30$ of the final electron density map. The part of the molecular skeleton lying between $x/a = 6/30$ and $10/30$ is shown. First contour (broken) at ½′ eÅ^{-3}; contour interval 1 eÅ^{-3}. 55 Contours omitted in the bromine peak.

DISCUSSION

(i) *General*

The structure as determined from the X ray analysis (Fig. 2) is in complete accord with the chemical and spectroscopic properties of the compound. The structure contains a courmarin system, a hydroxyl group, two ether oxygens and a gem dimethyl

group. The ten carbon atoms outside the coumarin system form two isopentyl chains linked together so as to yield a cyclohexane system and three methyl groups.

(ii) *Bond lengths*

The final atomic coordinates are given in Table 1 and bond lengths calculated from them are contained in Table 2. The standard deviations, which are also given in Table 2, were calculated using Cruickshank's formulae[11].

The mean carbon-carbon bond distance in the coumarin system is close to the standard aromatic value of 1·395 Å and none of the individual values differ significantly from either the mean or this standard value. The O_1—C_2 and O_1—C_6 bonds are close to the usual heterocyclic distance of 1·37 Å, the C_2—O_{20} bond is close to the carbonyl distance and the B_r—C_7 bond does not differ significantly from that of a normal single bond. Outside the coumarin system, however, the agreement is not so good, carbon-carbon single bonds varying in length from 1·32 Å to 1·76 Å compared with the standard value of 1·54 Å.

These discrepancies can be readily attributed to the action of inverse overlap. Srinivasan[12] has shown that, where a noncentrosymmetric structure contains a heavy atom or group of atoms with an approximate centre of symmetry, there is a strong interaction between the parameters of an atom and those of another atom related to it by inversion through this centre. This invalidates the assumption, made in the block diagonal approximation to the least squares matrix, that the off-diagonal terms representing these interactions are negligible. This effect, which amounts to the overlapping of an atom with its inverse, severely limits the precision of the positional parameters of these atoms.

To relate this phenomenon to bromo-bruceol, consider, firstly, the bromine and other atoms which lie close to the $x = \frac{1}{4}$ plane. An atom with coordinates $(\frac{1}{4} + \delta x, y, z)$ transforms under the two-fold rotor about a to $(\frac{1}{4} + \delta x, \bar{y}, \bar{z})$. The inverse of the latter point through $(\frac{1}{4}, 0, 0)$ is $(\frac{1}{4} - \delta x, y, z)$ so $(\frac{1}{4}, 0, 0)$ is an approximate centre of symmetry for all the atoms lying near the $x = \frac{1}{4}$ plane. The "inverse overlap" of these atoms gives rise to probable errors in their positions which are considerably greater than those indicated by the usual standard deviation formulae. Only the x coordinates will be affected in this instance, however, as the overlap is exact in the y and z directions.

Now consider the atoms C_{13} and C_{14} which have approximately the same y and z coordinates as C_{18} and C_{19} respectively and which also lie at approximately the same distance from, but on the opposite side of, the $x = \frac{1}{4}$ plane. By a similar argument to the above, it can be shown that an atom at (x_1, y_1, z_1) will overlap with the inverse of the symmetry repeat of another atom at (x_2, y_2, z_2) if $x_2 \simeq \frac{1}{2} - x_1$, $y_2 \simeq y_1$ and $z_2 \simeq z_1$. This is precisely the case for the above-mentioned pairs of atoms. The overlap is not necessarily exact in any direction so all the coordinates of these atoms are unreliable.

All the bond lengths where the agreement is poor either involve one of the atoms C_{13}, C_{14}, C_{18}, C_{19} or are strongly dependent on the x coordinates of an atom lying near the $x = \frac{1}{4}$ plane. The discrepancies can therefore be attributed to inverse overlap.

According to Srinivasan,[12] inverse overlap should not directly affect the thermal

[11] D. W. J. Cruickshank, *Acta Cryst.* **2**, 65 (1949).

[12] R. Srinivasan, *Acta Cryst.* **14**, 1163 (1961).

TABLE 1. ATOMIC COORDINATES

(a) Carbon, Oxygen and Bromine

Atoms	*x/a*	*y/b*	*z/c*
O_1	0·2415	0·2994	0·2100
C_2	0·2275	0·3678	0·1927
C_3	0·2575	0·4300	0·2261
C_4	0·2718	0·4136	0·2778
C_5	0·2598	0·3409	0·2948
C_6	0·2383	0·2843	0·2605
C_7	0·2644	0·2059	0·2744
C_8	0·3088	0·1912	0·3235
C_9	0·3476	0·2485	0·3572
C_{10}	0·3033	0·3226	0·3455
O_{11}	0·2867	0·3750	0·3793
C_{12}	0·2617	0·3516	0·4313
C_{13}	0·4288	0·2943	0·4476
C_{14}	0·4269	0·2223	0·4166
C_{15}	0·2128	0·1810	0·4248
C_{16}	0·2374	0·1064	0·3924
O_{17}	0·3081	0·1200	0·3430
C_{18}	0·0917	0·3055	0·4380
C_{19}	0·0922	0·2331	0·4129
O_{20}	0·2246	0·3800	0·1478
C_{21}	0·2566	0·4194	0·4628
O_{22}	0·6094	0·3246	0·4423
C_{23}	0·3502	0·0459	0·4161
C_{24}	0·0135	0·0822	0·3838
Br_{25}	0·2399	0·1316	0·2268

(b) Hydrogen

Atom	"Bonding Atom"*	*x/a*	*y/b*	*z/c*
H_{26}	C_3	0·2170	0·4833	0·2153
H_{27}	C_4	0·2900	0·4590	0·3050
H_{28}	C_{18}	0·1029	0·2839	0·4740
H_{29}	C_{18}	−0·0044	0·3417	0·4253
H_{30}	C_{13}	0·3870	0·2700	0·4800
H_{31}	C_{19}	0·1187	0·2500	0·3730
H_{32}	C_{19}	−0·0330	0·2212	0·4193
H_{33}	C_{14}	0·5330	0·2049	0·4203
H_{34}	C_{15}	0·2370	0·1670	0·4703
H_{35}	C_{21}	0·3816	0·4336	0·4737
H_{36}	C_{21}	0·1726	0·4600	0·4510
H_{37}	C_{21}	0·2056	0·4131	0·5100
H_{38}	O_{22}	0·6145	0·3841	0·4210
H_{39}	C_{23}	0·4897	0·0750	0·4410
H_{40}	C_{23}	0·4170	0·0130	0·3860
H_{41}	C_{23}	0·2730	0·0280	0·4470
H_{42}	C_{24}	0·0800	0·0330	0·3659
H_{43}	C_{24}	−0·0214	0·1429	0·3628
H_{44}	C_{24}	0·0170	0·0730	0·4220

* "Bonding Atom" indicates that atom to which the hydrogen atom is bonded.

TABLE 2. BOND LENGTHS

Atoms	Bond (Å)	σ(Å)	Atoms	Bond (Å)	σ(Å)
O_1—C_2	1·31	0·04	C_{13}—C_{13}	1·65	0·05
C_2—C_3	1·44	0·05	C_{13}—C_{14}	1·53	0·05
C_3—C_4	1·42	0·05	C_{14}—C_{15}	1·74	0·05
C_4—C_5	1·38	0·05	C_{15}—C_{16}	1·60	0·05
C_5—C_6	1·38	0·05	C_{16}—O_{17}	1·44	0·04
C_6—O_1	1·38	0·04	O_{17}—C_8	1·38	0·04
C_2—O_{20}	1·22	0·04	C_9—C_{14}	1·76	0·05
C_6—C_7	1·46	0·05	C_{12}—C_{18}	1·50	0·05
C_7—C_8	1·38	0·05	C_{18}—C_{19}	1·46	0·05
C_8—C_9	1·40	0·05	C_{19}—C_{15}	1·32	0·05
C_9—C_{10}	1·40	0·05	C_{13}—C_{21}	1·48	0·05
C_{10}—C_5	1·43	0·05	C_{13}—O_{22}	1·40	0·04
C_7—Br_{25}	1·85	0·03	C_{16}—C_{23}	1·50	0·05
C_{10}—O_{11}	1·31	0·04	C_{16}—C_{24}	1·70	0·05
O_{11}—C_{12}	1·47	0·04			

The standard deviations (σ) were calculated using Cruickshank's (1949) formulae. Distances involving hydrogen atoms are not given.

parameters. However, if the atomic coordinates are inaccurate the thermal parameters may well refine incorrectly to compensate for this. This, in conjunction with the poor quality of the intensity data, is taken as the reason for the divergence of the temperature parameters during the least squares refinement.

(iii) *Intermolecular contacts and packing*

The shorter intermolecular distances (Fig. 7) are consistent with Van der Waals contacts, apart from the O - - - O contact between the hydroxyl oxygen of one molecule and the carbonyl of another. This contact is 2·89 Å long and appears to be a hydrogen bond. Infrared measurements on solutions of bruceol indicate the presence of an intramolecular hydrogen bond involving the hydroyl group, but not the carbonyl. The only oxygen in the same molecule anywhere near the correct distance from O_{22} is O_{11}, which is at a distance of 3·00 Å. However, the angle C_{13}–O_{22} - - - O_{11} is 58°, so an improbably large distortion of the molecule would be required for the atom to point towards O_{11}. It is unlikely that bromination would affect the internal hydrogen bond system and it is difficult to imagine any reorientation of the molecule, when in solution, which would permit the formation of an intramolecular hydrogen bond.

Each molecule consists of an approximately planar coumarin ring system with the cyclohexane system nearly at right angles to it (Fig. 7). Two molecules are packed, with their coumarin systems overlapping, into the part of the cell bounded by $x = 0$, a; $y = 0$, b/2; and $z = 0$, c/2. The repetition of this unit by the symmetry operations gives a tightly packed system.

(iv) *Planarity and distortions*

The equations for the mean planes through various parts of the molecular were calculated by least squares methods and are given, along with out-of-plane deviations,

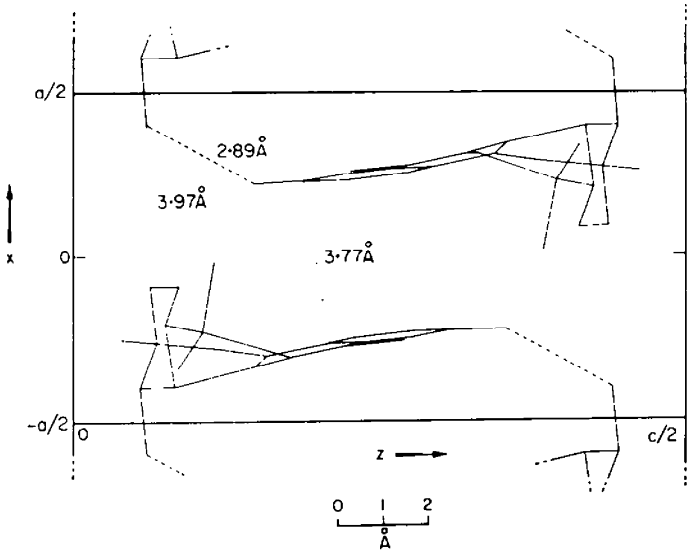


FIG. 7. A projection down *b* of two molecules related by the two-fold rotor parallel to *b*, illustrating the packing. Broken lines show hydrogen bonds and dotted lines some typical Van der Waals contacts.

in Table 3. Plane I is the mean plane through the whole coumarin system, plane II is that through atoms 1 to 6 and 20, and plane III is that through the benzene ring. The deviations from plane II are less than 0·1 Å and those from plane III are less than 0·07 Å, none of them being significant when compared with the standard deviations in the atomic positions of 0·04 Å. However, the planes themselves are inclined

TABLE 3. MEAN PLANES AND DEVIATIONS

Plane	Atoms	Equation
I	1 to 10 and 20	$0{\cdot}431x + 0{\cdot}052y - 0{\cdot}081z = 1$
II	1 to 6 and 20	$0{\cdot}946x - 0{\cdot}043y - 0{\cdot}067z = 1$
III	5 to 10	$1{\cdot}038x + 0{\cdot}115y - 0{\cdot}208z = 1$

Atom	δ(I)	δ(II)	δ(III)
O_1	0·16	0·05	—
C_2	0·09	0·07	—
C_3	0·22	0·03	—
C_4	0·23	0·05	—
C_5	0·03	0·01	0·02
C_6	0·27	0·05	0·06
C_7	0·27	—	0·10
C_8	0·03	—	0·07
C_9	0·34	—	0·11
C_{10}	0·19	—	0·03
O_{20}	0·04	0·02	—

δ refers to the perpendicular deviation from the plane, expressed in Å.

at $11\frac{1}{2}°$ to each other, indicating that the coumarin system as a whole is slightly buckled. This is supported by the calculated deviations from plane I which are as great as 0·34 Å. The degree of buckling cannot be assessed with certainty, however, as the deviations are strongly dependent on the x coordinates of the atoms which are all adversely affected by inverse overlap.

DEGRADATION

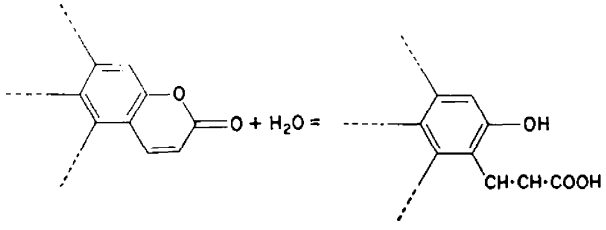
The degradation of the molecule, which occurs steadily with time, but is accompanied neither by changes in the systematic absences, not significant variations in the cell parameters, gives rise to the following phenomena:

(a) The relative intensities of some of the reflections in all regions of reciprocal space change with time.

(b) The mean isotropic temperature coefficient is larger for data collected with older crystals.

(c) A number of atomic peaks, but in particular C_3, are significantly lower in height than expected. The degradation presumably results from chemical action at these points.

The most probable course of the reaction involves a spontaneous combination with atmospheric water vapour as follows:



This reaction takes place in solution in the presence of alkali, although it is then accompanied by a number of side reactions. The above re-arrangement could occur without requiring any major change in cell size, but it is difficult to see how this could fail to reduce the peak height at C_2, whereas no such reduction was observed during the structure analysis.

In order to enable a more detailed investigation into the mechanism of degradation to be made, the (O k l) intensity data was recollected using older crystals. Using the results of a Wilson plot, the data was then multiplied by a modification function which gave it the same mean temperature factor and scale as the original (O k l) data obtained from a fresh crystal. Using the phases from the least squares refinement and the differences between the two sets of data as coefficients, a synthesis, projected on (1 0 0) was evaluated. This indicated that a number of the atoms had undergone substantial shifts during the degradation, but, unfortunately, the overlap prevented any interpretation of these features in the most interesting part of the molecule, and no significant information was obtained.

The degradation mechanism could probably be determined from fairly accurate three-dimensional analyses of both fresh and degraded crystals. However, the experimental difficulties involved both in collecting this kind of data and in overcoming the problem of inverse overlap in the refinement appear formidable and no further work is contemplated at this stage.

NUCLEAR MAGNETIC RESONANCE STUDY

After the structure of bruceol had been determined a study of the N.M.R. spectrum was made and interpreted by Dr. J. N. Shoolery of Varian Associates. The results were obtained at 60 megacycles using tetramethyl silane as internal standard in deuterochloroform solution. Two doublets at $\delta = 7{\cdot}90$ and $6{\cdot}1$ p.p.m. have a coupling constant of approximately 10 c.p.s. and may be assigned to protons α and β to the coumarin carbonyl respectively. A sharp line at $\delta = 6{\cdot}4$ arises from the lone aromatic proton. A pair of doublets at $\delta = 3{\cdot}85$ shows spin coupling to a doublet at $\delta = 2{\cdot}60$ which collapses on addition of hydrogen chloride, requiring that the signals near 3·85 p.p.m. arise from the proton on the same carbon as the hydroxyl

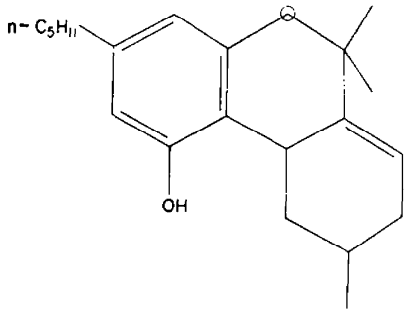


FIG. 8

group and the doublet at $\delta = 2{\cdot}60$ arise from the hydroxyl group. A closely spaced triplet at $\delta = 2{\cdot}90$ is assigned to the benzylic proton and the weak couplings observed are in agreement with its equatorial disposition. The three methyl groups are present as singlets at $\delta = 1{\cdot}10$, $1{\cdot}50$ and $1{\cdot}60$ p.p.m.

Bruceol may be regarded as a $C_6 - C_3$ unit based on phloroglucinol combined with two isopentyl units. Other combinations of these units which are known to occur in the genus *Eriostemon* are eriostoic[1] and eriostemoic acids.[2] Again bruceol has analogy to tetrahydrocannabinol (Fig. 8) in the attachment of the terpene unit to the benzenoid system. Whalley[13] has suggested biogenesis of terathydrocannabinol by condensation of geranyl pyrophosphate with 5-*n*-amylresorcinol with subsequent cyclization of the geranyl group leading to cannabinol. A related process can be envisaged for the formation of bruceol and might proceed via the geranyl coumarin, ostruthin, known to occur in the related species Eriostemon tomentellus.[2]

EXPERIMENTAL

Extraction of Eriostemon brucei

Crushed leaves and terminal branches (9 kg) collected at Pindar, Western Australia were extracted with ether (12 l.) during 7 days at room temp. The solvent was separated and partitioned with 5% hydrochloric acid, 8% sodium bicarbonate and 5% sodium hydroxide solution. The acid extract was basified with sodium bicarbonate and the precipitate (15 g) separated and crystallized from methanol to give maculosidine (8·0 g), m.p. and mixed m.p. 186·5° (Found: C, 65·2; H, 5·1; N, 5·4; OMe, 34·2; Calc. for $C_{14}H_{13}O_4N$: C, 64·9; H, 5·1; N, 5·4; OMe, 35·9%). The sodium bicarbonate extract was acidified and the precipitate separated, washed with a little ether and crystallized from aqueous-ethanol as cream *needles*, m.p. 230°, $[\alpha]_D$ −130° (C_2H_5OH) (Found: C, 65·9; H, 6·5; $C_{19}H_{23}O_6$

[13] W. B. Whalley, in W. D. Ollis, *Chemistry of Natural Phenolic Compounds*, p. 38. Pergamon Press, London (1961).

requires: C, 65·9; H, 6·4%). The neutral extract was concentrated until precipitation occurred (400 ml) and after 24 hr the precipitate (15 g) was collected and crystallized from methyl ethyl ketone and then ethanol to give *bruceol* as prisms, m.p. 201°, $[\alpha]_D$ −297° ($CHCl_3$) (Found: C, 69·1; H, 6·2; O, 24·5%. $C_{19}H_{20}O_5$ requires: C, 69·5; H, 6·1; O, 24·4%). The residual ether was evaporated and the residue steam distilled. The essential oil (210 g) had d_4^{20} 0·9100; n_D^{20}, 1·4650; $[\alpha]_D$+2°. Gas chromatography showed it to be mainly cineole and monoterpenes.

The *acetate* was prepared with acetic anhydride in pyridine. Crystallization from di-isopropyl ether gave needles, m.p. 175°, $[\alpha]_D$ −357° ($CHCl_3$) (Found: C, 68·4; H, 6·2; O, 25·7; $C_{21}H_{22}O_6$ requires: C, 68·1; H, 6·0; O, 25·9%).

The *benzoate* prepared in pyridine, crystallized from ethanol as cubes, m.p. 221°, $[\alpha]_D$ −360° ($CHCl_3$) (Found: C, 71·8; H, 5·7; $C_{26}H_{24}O_6$ requires: C, 72·2; H, 5·6%).

The p-*nitrobenzoate* was prepared in pyridine and crystallized from ethanol as cream needles, m.p. 197°, $[\alpha]_D$ −370° ($CHCl_3$) (Found: C, 65·8; H, 5·0; N, 3·0; $C_{26}H_{23}O_8N$ requires: C, 65·4; H, 4·9; N, 2·9%). All these derivatives were transparent in the hydroxyl region of the I.R. spectrum.

Hydrolysis of bruceol

Bruceol (1 g) was dissolved in boiling 10% aqueous potassium hydroxide and after 1 hr the solution was diluted, acidified with hydrochloric acid and extracted with chloroform. The acids (0·2 g) were recovered by washing with 8% sodium bicarbonate solution and crystallized from aqueous ethanol as cream needles, m.p. 230° (Found: C, 66·2; H, 6·6; Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·4%), alone or mixed with the acid isolated from the plant material. The acid failed to cyclize on heating alone or with acid solutions. The neutral fraction (0·55 g) was identified as bruceol by conversion to the acetate, m.p. and mixed m.p. 175°.

Dihydrobruceol

Bruceol (0·17 g) in ethanol (15 ml) was hydrogenated with platinum oxide for 6 hr (1 mole of hydrogen). Crystallization of the product from acetone-light petroleum gave prisms, m.p. 215°, $[\alpha]_D$ −7° ($CHCl_3$) (Found: C, 69·1; H, 6·4; $C_{19}H_{22}O_6$ requires: C, 69·1; H, 6·7%). The *acetate*, prepared in pyridine, crystallized from benzene-light petroleum as prisms, m.p. 187–190° $[\alpha]_D$ −74° ($CHCl_3$) (Found: C, 67·3; H, 6·4; $C_{21}H_{24}O_6$ requires: C, 67·7; H, 6·5%). This latter derivative was transparent in the hydroxyl region of the I.R. spectrum.

Chromic acid oxidation of bruceol

Bruceol (0·39 g) in acetic acid (10 ml) was treated with a solution of chromic acid (1·0 g) in water (5 ml) during 18 hr at room temp. Basification of the reaction mixture and distillation into a saturated solution of 2,4-dinitrophenylhydrazine in 10% sulphuric acid and crystallization of the precipitate (0·04 g) from ethanol gave acetone 2,4-dinitrophenylhydrazone as yellow needles m.p. 124–125°, undepressed on admixture with an authentic sample of m.p. 127°.

Bruceol monobromide

Bruceol (0·2 g) in dioxan (10 ml) containing sodium carbonate (0·18 g) was treated with a solution of bromine (1 g) in dioxan (25 ml) until a yellow colour persisted for 15 min. The reaction mixture was diluted with water and the product (0·26 g) isolated with chloroform. Crystallization from benzene gave bruceol monobromide as needles m.p. 184–185° (dec.), $[\alpha]_D$ −291° ($CHCl_3$) (Found: 56·2; H, 4·8; Br, 21·3; $C_{19}H_{19}O_5Br$ requires: C, 56·0; H, 4·7; Br, 19·6%).

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