The Structure of 3β-Acetoxy-15α-(o-nitrobenzoyl)oxy-5β,14β-bufa-8,20,22-trienolide

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Abstract

The title bufadienolide, $C_{33}H_{37}NO_8$, $M_r = 575.66$, is orthorhombic, space group $P2_12_12_1$, with $a = 31.53$ (2), $b = 11.533$ (6), $c = 8.221$ (4) Å, $Z = 4$, $D_m = 1.27$, $D_c = 1.28$ Mg m^{-3}, μ(Mo $K\alpha$) = 0.054 mm^{-1}, $F(000) = 1224$. The structure was solved by direct methods in which the required additional phase information was only obtained by the examination of a centrosymmetric projection. Refinement by least squares yielded a final $R = 0.095$ for 1383 observed reflections. There is *cis* fusion of the A/B and C/D rings, while the B/C rings are fused by a double bond.

Introduction

The bufadienolide class of naturally occurring steroids are defensive components of certain toad (Ode, Pettit & Kamano, 1975) and firefly (Meinwald, Wiemer & Eisner, 1979) venoms and some of these interesting substances have been found to inhibit significantly growth of the US National Cancer Institute's cell line derived from a human nasopharynx carcinoma (KB) and murine P388 lymphocytic leukæmia (Kamano, Pettit, Inoue, Tozawa & Komeichi, 1977). For the purpose of eventually developing a useful synthetic approach to certain 11-oxygenated bufadienolides it became necessary to define completely the product resulting from selenium dioxide dehydrogenation of 14-dehydrobufalin. The title bufadienolide was the major event of this reaction and a crystal structure determination now follows.

Experimental

The compound (m.p. 450–452 K) was recrystallized from acetone. The crystals were colourless needles elongated along **b**. Preliminary photographs (Cu $K\alpha$ radiation, $\lambda = 1.542$ Å) indicated the space group

$P2_12_12_1$. Crystal data are given in the *Abstract*. The lattice constants were obtained by least squares from the settings of 25 reflections measured on a four-circle diffractometer with graphite-monochromated Mo $K\alpha$ radiation ($\lambda = 0.7107$ Å); the data were corrected for Lorentz–polarization effects but not for absorption.

Structure determination and refinement

An initial attempt to solve the structure in the conventional manner using the multisolution tangent refinement procedure of *SHELX* was unsuccessful. A convergence map (Germain, Main & Woolfson, 1971) calculated for reflections with $E > 1.2$ in the data set showed many path breaks and weak links in the first 50 reflections in the pathway. The subsequent figures of merit for selecting the 'best' solutions ranged from $R_A = 0.126$ to 0.149, and no interpretable E maps were obtained.

The conventional use of *MULTAN* was also unsuccessful; 'combined' figure-of-merit values ranging from 2.342 to 1.153 again yielded no discernible molecular fragments.

The structure was ultimately obtained by first examining a centrosymmetric zone and by using the phases so established in the starting set of a three-dimensional analysis (Boeyens, 1977).

The (001) projection was chosen because the c axis is the shortest. In order to have the symmetry element in projection at the origin, the symmetry operations were translated through $\frac{1}{4}00$.

The centrosymmetric direct-methods package of *SHELX*76 (Sheldrick, 1978) was then used to generate four two-dimensional E maps from the $hk0$ reflections, with the E limit set low at 0.8 in order to include a reasonable number of reflections (50). The four maps corresponded to four possible sets of sign combinations for the origin and multisolution reflections. A three-dimensional convergence map was then calculated using as a starting set those $hk0$ reflections which had been obtained with their phases fixed according to the first combination. Included in the

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Table 1. *Experimental and refinement parameters*

Crystal dimensions (mm)	0·68 × 0·18 × 0·18
Scan mode	ω–2θ
Scan width (deg θ)	0·9
Scan speed (deg θ s⁻¹)	0·03
Range scanned (2θ) (°)	6–46
Stability of standard reflections (%)	1·2
Number of reflections collected	2411
Number of 'observed' reflections	1383 with $I_{(rel)} > 2\sigma I_{(rel)}$
Number of variables	180
$R = \sum ||F_o| - |F_c||/\sum |F_o|$	0·0952
$R_w = \sum w^{1/2}||F_o| - |F_c||/\sum w^{1/2} |F_o|$	0·0758
Weighting scheme w	$(\sigma^2 F)^{-1}$
U (methyl H) (Å²)	0·106
U (aromatic, methylene and methine H) (Å²)	0·064

Table 2. *Fractional atomic coordinates* (×10³) *and isotropic temperature factors* (Å² ×10³) *of the heavy atoms*

	x	y	z	U_{iso}
C(1)	645 (3)	7777 (10)	9975 (14)	55 (4)
C(2)	1056 (4)	8426 (9)	10169 (16)	65 (4)
C(3)	1042 (4)	9646 (10)	9572 (14)	62 (4)
C(4)	884 (3)	9661 (9)	7805 (13)	45 (4)
C(5)	458 (3)	9043 (10)	7522 (13)	45 (4)
C(6)	357 (3)	9012 (10)	5673 (13)	54 (4)
C(7)	687 (4)	8331 (9)	4748 (14)	56 (4)
C(8)	809 (3)	7220 (9)	5511 (13)	40 (3)
C(9)	721 (3)	6955 (10)	7107 (14)	42 (3)
C(10)	465 (3)	7762 (10)	8165 (14)	46 (3)
C(11)	847 (4)	5773 (10)	7759 (15)	58 (4)
C(12)	770 (4)	4856 (10)	6472 (14)	59 (4)
C(13)	1028 (3)	5075 (10)	4913 (15)	55 (4)
C(14)	1076 (3)	6402 (9)	4499 (14)	44 (3)
C(15)	1549 (3)	6683 (9)	4436 (13)	39 (3)
C(16)	1757 (3)	5521 (8)	4051 (12)	33 (3)
C(17)	1503 (3)	4678 (8)	5124 (14)	37 (3)
C(18)	820 (4)	4465 (11)	3436 (16)	71 (4)
C(19)	7 (3)	7299 (10)	8292 (14)	50 (4)
C(20)	1585 (3)	3382 (10)	4836 (16)	47 (4)
C(21)	1591 (4)	2690 (11)	6115 (17)	63 (4)
C(22)	1648 (4)	2895 (11)	3275 (16)	61 (4)
C(23)	1696 (4)	1721 (11)	3090 (17)	63 (4)
C(24)	1666 (5)	1047 (14)	4467 (22)	98 (6)
C(25)	709 (5)	11385 (13)	10644 (18)	80 (5)
C(26)	346 (4)	11740 (13)	11752 (18)	94 (5)
C(27)	2061 (4)	7566 (9)	6184 (14)	42 (3)
C(28)	2142 (3)	7903 (10)	7958 (14)	45 (4)
C(29)	2181 (3)	7084 (10)	9196 (14)	45 (4)
C(30)	2245 (3)	7407 (10)	10810 (14)	50 (4)
C(31)	2260 (4)	8565 (10)	11168 (16)	58 (4)
C(32)	2225 (3)	9377 (10)	9972 (13)	50 (4)
C(33)	2166 (3)	9071 (10)	8338 (14)	43 (3)
O(3)	712 (2)	10205 (7)	10618 (10)	61 (3)
O(15)	1681 (2)	7043 (6)	6069 (8)	38 (2)
O(21)	1637 (3)	1491 (8)	6011 (12)	86 (3)
O(24)	1704 (3)	−51 (8)	4478 (12)	96 (3)
O(25)	945 (3)	11984 (8)	9903 (13)	107 (4)
O(27)	2295 (2)	7783 (6)	5087 (9)	55 (2)
N(29)	2168 (3)	5810 (10)	8810 (16)	74 (4)
O(291)	2316 (2)	5506 (7)	7537 (10)	69 (3)
O(292)	1984 (3)	5197 (8)	9783 (12)	101 (4)

starting set were selected fixed reflections obtained from the first attempt at solution of the structure using *SHELX*, with their phases adjusted according to the shifted symmetry parameters. The starting set was then used in the normal way. Of the 12 *E* maps calculated from the various permutations generated, the first (having a figure of merit $R_A = 0·118$) yielded 41 of the heavy atoms. Least-squares refinement and a subsequent weighted difference synthesis gave the position of the remaining C atom. In the final refinement, the heavy atoms were treated isotropically; the H atoms were constrained to be 1·08 Å from the corresponding C atoms, their positions being dictated by the geometry of the molecule. The H atoms in the methyl groups were refined as rigid groups. The isotropic temperature factors of the H atoms were refined as two single parameters: one for methyl H atoms and the other for the remaining H atoms. Their final values are reported in Table 1. Fractional atomic coordinates and thermal parameters are listed in Table 2.

Results and discussion

A perspective view of the molecule with the salient atomic nomenclature is shown in Fig. 1. Bond lengths, angles and selected torsion angles are given in Tables 3 and 4.*

The purpose of this investigation was to ascertain the configuration at C(14) and C(15), and we determined that H(14) is β and the (o-nitrobenzoyl)oxy substituent at C(15) is α. There is *cis* fusion of the *A/B* and *C/D* rings while the *B/C* rings are fused by a double bond; C(8)—C(9) = 1·38 (1) Å. The bond lengths and angles are in good agreement with accepted values.

We usually describe the molecular conformation of the individual steroid rings by the calculation of
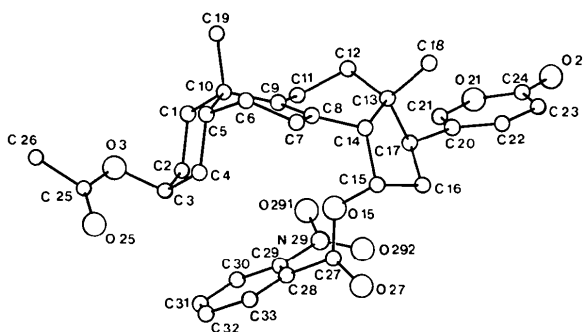
Fig. 1. The molecular structure and salient atomic nomenclature.

asymmetry parameters, as popularized by Duax & Norton (1975) in their review of steroid structure. Recently, however, Boeyens (1978) has described a method of calculating parameters of pucker in the form of polar coordinates. The total degree of pucker is described by the radial coordinate Q, and the angular coordinates θ and φ (six-membered rings), or φ (five-membered rings) describe the shape of the ring. For the conformational nomenclature, the atoms in

Table 3. Bond lengths (Å) and angles (°)

C(1)–C(2)	1·51 (1)		C(16)–C(17)	1·54 (1)
C(1)–C(10)	1·59 (1)		C(17)–C(20)	1·54 (1)
C(2)–C(3)	1·49 (1)		C(25)–C(26)	1·52 (2)
C(3)–C(4)	1·54 (1)		C(25)–O(3)	1·36 (1)
C(3)–O(3)	1·50 (1)		C(25)–O(25)	1·18 (2)
C(4)–C(5)	1·54 (1)		C(27)–C(28)	1·53 (1)
C(5)–C(6)	1·55 (1)		C(27)–O(15)	1·35 (1)
C(5)–C(10)	1·57 (1)		C(27)–O(27)	1·19 (1)
C(6)–C(7)	1·51 (1)		C(20)–C(21)	1·32 (2)
C(7)–C(8)	1·48 (1)		C(20)–C(22)	1·42 (1)
C(8)–C(9)	1·38 (1)		C(21)–O(21)	1·39 (1)
C(8)–C(14)	1·51 (1)		C(24)–C(23)	1·38 (2)
C(9)–C(10)	1·51 (1)		C(24)–O(21)	1·37 (2)
C(9)–C(11)	1·52 (1)		C(24)–O(24)	1·27 (2)
C(10)–C(19)	1·54 (1)		C(23)–C(22)	1·37 (1)
C(11)–C(12)	1·52 (1)		C(28)–C(29)	1·39 (1)
C(12)–C(13)	1·54 (1)		C(28)–C(33)	1·39 (1)
C(13)–C(14)	1·58 (1)		C(29)–C(30)	1·39 (1)
C(13)–C(17)	1·58 (1)		C(29)–N(29)	1·50 (1)
C(13)–C(18)	1·55 (1)		C(30)–C(31)	1·37 (1)
C(14)–C(15)	1·53 (1)		C(31)–C(32)	1·36 (1)
C(15)–C(16)	1·53 (1)		C(32)–C(33)	1·40 (1)
C(15)–O(15)	1·47 (1)		N(29)–O(291)	1·20 (1)
			N(29)–O(292)	1·22 (1)

C(2)–C(1)–C(10)	114 (1)		C(16)–C(15)–C(16)	108 (1)
C(1)–C(2)–C(3)	114 (1)		C(15)–C(16)–C(17)	102 (1)
C(2)–C(3)–C(4)	109 (1)		C(13)–C(17)–C(16)	104 (1)
C(2)–C(3)–O(3)	104 (1)		C(13)–C(17)–C(20)	115 (1)
C(4)–C(3)–O(3)	108 (1)		C(16)–C(17)–C(20)	116 (1)
C(3)–C(4)–C(5)	115 (1)		C(26)–C(25)–O(3)	107 (1)
C(4)–C(5)–C(6)	110 (1)		C(26)–C(25)–O(25)	129 (1)
C(4)–C(5)–C(10)	112 (1)		O(3)–C(25)–O(25)	125 (1)
C(6)–C(5)–C(10)	108 (1)		C(28)–C(27)–O(15)	109 (1)
C(5)–C(6)–C(7)	111 (1)		C(28)–C(27)–O(27)	124 (1)
C(6)–C(7)–C(8)	115 (1)		O(15)–C(27)–O(27)	126 (1)
C(7)–C(8)–C(9)	123 (1)		C(17)–C(20)–C(21)	118 (1)
C(7)–C(8)–C(14)	117 (1)		C(17)–C(20)–C(22)	123 (1)
C(9)–C(8)–C(14)	120 (1)		C(21)–C(20)–C(22)	119 (1)
C(8)–C(9)–C(10)	121 (1)		C(20)–C(21)–O(21)	124 (1)
C(8)–C(9)–C(11)	119 (1)		C(23)–C(24)–O(21)	124 (1)
C(10)–C(9)–C(11)	119 (1)		C(23)–C(24)–O(24)	124 (1)
C(1)–C(10)–C(5)	108 (1)		O(21)–C(24)–O(24)	112 (1)
C(1)–C(10)–C(9)	111 (1)		C(24)–C(23)–C(22)	117 (1)
C(5)–C(10)–C(9)	113 (1)		C(20)–C(22)–C(23)	121 (1)
C(1)–C(10)–C(19)	106 (1)		C(27)–C(28)–C(29)	123 (1)
C(5)–C(10)–C(19)	110 (1)		C(27)–C(28)–C(33)	118 (1)
C(9)–C(10)–C(19)	109 (1)		C(29)–C(28)–C(33)	119 (1)
C(9)–C(11)–C(12)	110 (1)		C(28)–C(29)–C(30)	122 (1)
C(11)–C(12)–C(13)	112 (1)		C(28)–C(29)–N(29)	120 (1)
C(12)–C(13)–C(14)	113 (1)		C(30)–C(29)–N(29)	118 (1)
C(12)–C(13)–C(17)	111 (1)		C(29)–C(30)–C(31)	118 (1)
C(14)–C(13)–C(17)	102 (1)		C(30)–C(31)–C(32)	121 (1)
C(12)–C(13)–C(18)	111 (1)		C(31)–C(32)–C(33)	122 (1)
C(14)–C(13)–C(18)	108 (1)		C(28)–C(33)–C(32)	118 (1)
C(17)–C(13)–C(18)	111 (1)		C(3)–O(3)–C(25)	116 (1)
C(8)–C(14)–C(13)	116 (1)		C(15)–O(15)–C(27)	116 (1)
C(8)–C(14)–C(15)	115 (1)		C(21)–O(21)–C(24)	116 (1)
C(13)–C(14)–C(15)	108 (1)		C(29)–N(29)–O(291)	117 (1)
C(14)–C(15)–C(16)	104 (1)		C(29)–N(29)–O(292)	116 (1)
C(14)–C(15)–O(15)	108 (1)		O(291)–N(29)–O(292)	126 (1)

Table 4. Internal torsional angles of the steroid skeleton (°)

C(10)–C(1)–C(2)–C(3)	55 (1)	
C(2)–C(1)–C(10)–C(5)	–51 (1)	
C(1)–C(2)–C(3)–C(4)	–54 (1)	
C(2)–C(3)–C(4)–C(5)	54 (1)	Ring A
C(3)–C(4)–C(5)–C(10)	–54 (1)	
C(4)–C(5)–C(10)–C(1)	50 (1)	
C(5)–C(6)–C(7)–C(8)	45 (1)	
C(6)–C(7)–C(8)–C(9)	–16 (1)	
C(7)–C(8)–C(9)–C(10)	4 (1)	
C(8)–C(9)–C(10)–C(5)	–21 (1)	Ring B
C(9)–C(10)–C(5)–C(6)	47 (1)	
C(10)–C(5)–C(6)–C(7)	–60 (1)	
C(8)–C(9)–C(11)–C(12)	–39 (1)	
C(9)–C(11)–C(12)–C(13)	60 (1)	
C(11)–C(12)–C(13)–C(14)	–36 (1)	
C(12)–C(13)–C(14)–C(8)	–9 (1)	Ring C
C(13)–C(14)–C(8)–C(9)	33 (1)	
C(11)–C(9)–C(8)–C(14)	–8 (1)	
C(14)–C(13)–C(17)–C(16)	–27 (1)	
C(15)–C(16)–C(17)–C(13)	43 (1)	
C(14)–C(15)–C(16)–C(17)	–41 (1)	Ring D
C(13)–C(14)–C(15)–C(16)	24 (1)	
C(17)–C(13)–C(14)–C(15)	2 (1)	

Table 5. Ring conformations of the steroid skeleton

	Asymmetry parameters	Pucker parameters
Ring A	$\Delta C_{s(3)} = 0.9°$	$Q = 0.006$ Å $\varphi = 188°$ $\theta = 1°$
Ring B	$\Delta C_{2(5-6)} = 3.7°$	$Q = 0.359$ Å $\varphi = 156°$ $\theta = 133°$
Ring C	$\Delta C_{2(11-12)} = 2.5°$	$Q = 0.553$ Å $\varphi = 327°$ $\theta = 105°$
Ring D	$\Delta C_{s(16)} = 2.3°$	$Q = 0.429$ Å $\varphi = 69°$

each ring are considered in a clockwise order starting with the lowest number in the ring. Table 5 shows the conformational analysis using both systems.

The packing of the molecules is unremarkable, there being no intermolecular close contacts less than 3·5 Å.

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Molecular Force-Field and X-ray Crystal Structures of Campanulin, a Comparison

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Abstract

Molecular-mechanics calculations of campanulin, an 81-atom pentacyclic triterpenoid oxide, yielded structure parameters in generally good agreement with experimental values from a crystallographic study [Mo (1977). *Acta Cryst.* B33, 641–649]. The r.m.s. deviations between calculated and observed bond lengths, valency angles and torsion angles are 0·009 Å, 1·1° and 2°, respectively. Comparison with the corresponding experimental torsion angles in a closely related molecule indicates that some of these parameters in campanulin may be appreciably influenced by crystal forces. The calculated molecular geometry is very similar to that obtained in another study based on a significantly different force field [Faber (1977). Private communication]. The results demonstrate the ability of force-field methods to refine quite accurately the geometry of strained polycyclic systems of such complexity. Two different minimum-energy conformations of the molecule have been explored. Both calculations favour that found in the crystal by an energy difference of 7·7–12·4 kJ mol^{-1} (1·85–2·95 kcal mol^{-1}).

Introduction

Molecular force-field (FF) calculations are being used increasingly as a method to evaluate for known molecules properties of both static and dynamic nature, *e.g.* geometry, energies, heat of formation, vibrational frequencies *etc.*, and to predict such properties for related classes of unknown molecules (Engler, Andose & Schleyer, 1973; Altona & Faber, 1974; Dunitz & Bürgi, 1975; Ermer, 1976). Several force fields have been parametrized with respect to data obtained from diffraction, spectroscopy and thermochemical measurements and applied on a multitude of relatively simple molecules, mainly hydrocarbons. Extensive bibliographic lists of areas for FF studies are given by Engler *et al.* (1973) and Altona & Faber (1974). Apparently, total-valence FF investigations of more complex systems are rather few, one notable exception being a comprehensive study of steroid structures by Romers, Altona, Jacobs & de Graaff (1974); Altona & Faber (1974) discuss some other examples.

Fused hydrocarbon ring systems constitute an interesting group of compounds for FF calculations. The high internal strain that usually characterizes these molecules is reflected in large local variations in the structure parameters that will put the predictive abilities of any force field to test. Previous crystallographic studies of two related triterpenoids, baccharis oxide (Mo, 1973) and campanulin (Mo, 1977), led to the conclusion that their ring structures are considerably strained, largely due to a number of 1,3-diaxial methyl groups. Close agreement in geometry of the common structure fragments of the two compounds suggested that intermolecular forces play a minor role in determining the molecular conformation.

Campanulin (III and Fig. 1) is related to the