

## Structure of Wightionolide: Chemical and X-Ray Analysis †

By **Balawant S. Joshi,\* Venkatesh N. Kamat,** and **Tuticorin R. Govindachari,** CIBA-GEIGY Research Centre, Goregaon, Bombay 400063, India  
**B. Ramachandra Pai,** Presidency College, Madras 5, India  
**Gopinath Kartha and Kuan Tee Go,** Centre for Crystallographic Research, Rosewell Park Memorial Institute, Buffalo, New York, N.Y. 14203, U.S.A.

Wightionolide isolated from the leaves of *Andrographis wightiana* Arn. ex Nees is shown to be 17,19,20-trihydroxy-5 $\beta$ ,8 $\alpha$ H,9 $\beta$ H,10 $\alpha$ -labd-13-en-16,15-olactone (XII). The molecular structure of the dibromo-triacetate (V) of wightionolide has been determined by X-ray diffraction studies.

From the stem and leaves of *Andrographis wightiana* Arn. ex Nees (Acanthaceae) Govindachari *et al.* isolated the flavones wightin, echioidinin, and, in low yield, a new compound wightionolide.<sup>1</sup> By extraction of the leaves of *A. wightiana* with chloroform we have now obtained this bitter principle in *ca.* 0.5% yield, and echioidin,<sup>2</sup> and we now report chemical and X-ray crystallographic evidence for the structure of wightionolide.

### Chemical and Spectroscopic Data

Wightionolide (I) (C<sub>20</sub>H<sub>32</sub>O<sub>5</sub>) shows an end absorption at  $\lambda_{\text{max}}$  214 nm (log  $\epsilon$  3.9) and i.r. bands at 3540, 3300, 3080 (OH), and 1735 cm<sup>-1</sup> ( $\alpha\beta$ -unsaturated- $\gamma$ -lactone). The presence of an  $\alpha\beta$ -unsaturated- $\gamma$ -lactone group was also shown by a positive Legal colour reaction<sup>3</sup> and the appearance of a vinyl proton at  $\delta$  7.38br (1H, s) and the adjacent methylene at 4.75br (2H, s) in its n.m.r. spectrum. Hydrogenation of wightionolide over Pd-C gave the dihydro-derivative (II) which did not give the Legal colour reaction. Its i.r. spectrum showed bands at 3540, 3300 (OH), and 1750 cm<sup>-1</sup> (saturated  $\gamma$ -lactone) and in the n.m.r. spectrum, the signals at  $\delta$  7.38 and 4.75 were absent.

† Presented at the Vth I.U.P.A.C. Symposium on the Chemistry of Natural Products, London, 1968.

<sup>1</sup> T. R. Govindachari, P. C. Parthasarathy, B. R. Pai, and P. S. Subramaniam, *Tetrahedron*, 1965, **21**, 3237.

<sup>2</sup> T. R. Govindachari, P. C. Parthasarathy, B. R. Pai, and P. S. Subramaniam, *Tetrahedron*, 1965, **21**, 3715.

Selenium dehydrogenation of wightionolide gave 1,2,5-trimethylnaphthalene isolated as the *sym*-trinitrobenzene adduct. On the basis of these data and on biogenetic grounds, wightionolide is assumed to possess the labdane skeleton. The diterpene lactone andrographolide (III) is present in a closely related species, *Andrographis paniculata* Nees.<sup>4,5</sup>

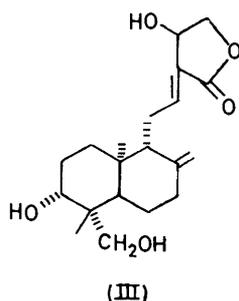
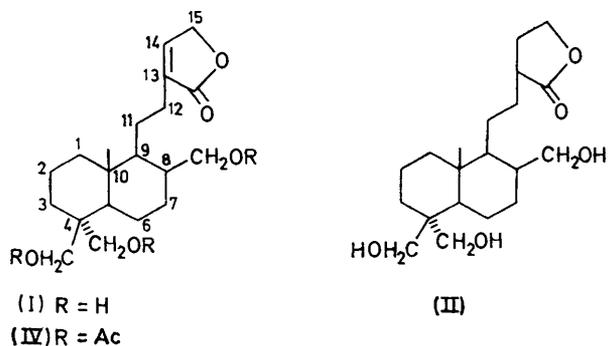
Acetylation of wightionolide gave a triacetate (IV), indicating that the remaining three oxygen atoms are present as hydroxy-groups. The triacetate on mild hydrolysis gave (I) indicating that there is no rearrangement of the molecule during acetylation. The n.m.r. spectrum of the triacetate showed signals at  $\delta$  7.1 (1H, :CHCH<sub>2</sub>), 4.75 (2H, :CHCH<sub>2</sub>), 4.1 (6H, m, CH<sub>2</sub>OAc), 2.03 (3H, OAc), and 2.04 (6H, OAc). The protons at  $\delta$  7.1 and 4.75 were shown to be mutually coupled by irradiation experiments. The 6H multiplet at  $\delta$  4.1 together with the evidence of the formation of a triacetate indicated that wightionolide contains three primary hydroxy-groups. Dihydrowightionolide (II) also gave a triacetate. On oxidation with chromium trioxide-pyridine, wightionolide gave an amorphous trialdehyde showing the aldehydic protons at  $\delta$  9.8,

<sup>3</sup> L. J. Haynes, in 'Modern Methods of Plant Analysis,' eds. K. Peach and M. V. Tracey, Springer-Verlag, Berlin, 1955, vol. II, p. 583.

<sup>4</sup> M. K. Gorter, *Rec. Trav. chim.*, 1911, **30**, 151.

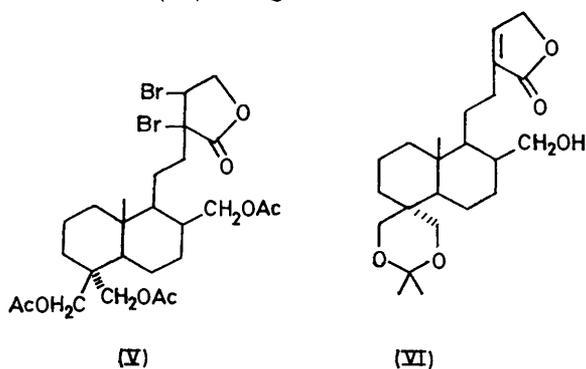
<sup>5</sup> M. P. Cava, W. R. Chan, R. P. Stein, and C. R. Willis, *Tetrahedron*, 1965, **21**, 2617.

9-6, and 9-3. The triacetate (IV) gave the dibromo-derivative (V). The bromo-compound reverted to (IV) on treatment with zinc and acetic acid. Suitable



crystals of (V) were used for the X-ray structure determination. In the n.m.r. spectrum, compounds (I) and (IV) showed the presence of only one tertiary methyl group, at  $\delta$  0.73 and 0.84 respectively, which should therefore be at either C-4 or C-10.

Wightionolide when pyrolysed with copper bronze gave formaldehyde, indicating the presence of a 1,3-glycol system.<sup>6</sup> Since the compound does not have a secondary hydroxy-group at C-3, the 1,3-glycol could comprise geminal hydroxymethyl groups at C-4. The existence of a 1,3-glycol system was also confirmed by the formation of a benzylidene derivative and a crystalline acetonide (VI). Wightionolide formed a ditrityl



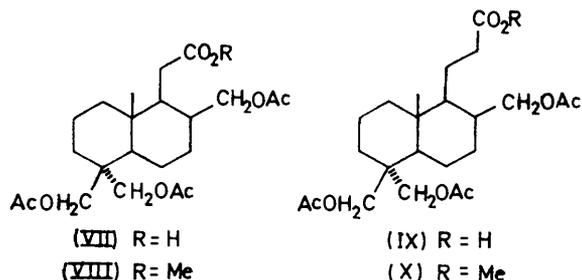
ether which on oxidation with chromic acid gave an amorphous aldehyde. This on treatment with sulphuric acid generated formaldehyde by a retro-aldol reaction.<sup>7,8</sup>

<sup>6</sup> F. E. King, T. J. King, and J. M. Ross, *J. Chem. Soc.*, 1954, 3995.

<sup>7</sup> D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 1954, 887.

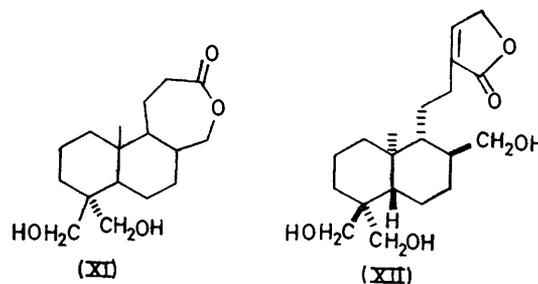
<sup>8</sup> M. P. Cava, W. R. Chan, L. J. Haynes, L. F. Johnson, and B. Winstein, *Tetrahedron*, 1962, **18**, 397.

Evidence regarding the mode of attachment of the  $\alpha\beta$ -unsaturated- $\gamma$ -lactone function in (I) was obtained from oxidation experiments. Potassium permanganate oxidation of (IV) gave a carboxylic acid (VII). Oxidation of (IV) with potassium permanganate-periodate reagent<sup>9</sup> gave a carboxylic acid, C<sub>23</sub>H<sub>36</sub>O<sub>8</sub> (IX), while



oxidation of wightionolide gave a lactone, C<sub>17</sub>H<sub>28</sub>O<sub>4</sub> (XI). Wightionolide is the only example of a bicyclic diterpene lactone of the labdane type possessing geminal hydroxymethyl groups at C-4. Diterpenes having the pimarane skeleton with both geminal methyls at C-4 being hydroxylated have been isolated from *Dacrydium colensoi*.<sup>10</sup>

On the basis of X-ray diffraction studies carried out on (V), wightionolide can be depicted by the stereo-structure (XII), without regard to the absolute configuration of the molecule.



#### X-Ray Structure Determination

The X-ray structure investigations were conducted at the Roswell Park Memorial Institute. The dibromotriacetate (V) of wightionolide used for the X-ray crystallographic studies formed colourless lath shaped crystals. Preliminary investigations indicated that these crystals are monoclinic.

*Crystal Data.*—C<sub>28</sub>H<sub>38</sub>Br<sub>2</sub>O<sub>8</sub>,  $M = 638$ . Monoclinic,  $a = 25.48$ ,  $b = 7.31$ ,  $c = 15.51$  Å,  $\beta = 99.72^\circ$ ,  $U = 2847$  Å<sup>3</sup>,  $D_m$  (by flotation) = 1.496 g cm<sup>-3</sup>,  $Z = 4$ . Systematic absences:  $0k0$  when  $k$  is odd, space group  $P2_1$ . Cu-K $\alpha$  radiation,  $\lambda = 1.5418$  Å;  $\mu(\text{Cu-K}\alpha) = 44.2$  cm<sup>-1</sup>.

The space group  $P2_1$  implies that there are two molecules of dibromowightionolide triacetate in the asymmetric unit. As these two molecules are crystallographically independent, this necessitates the de-

<sup>9</sup> R. Lemieux and E. von Rudolf, *Canad. J. Chem.*, 1955, **33**, 1701.

<sup>10</sup> R. M. Carman, R. E. Corbett, P. K. Grant, M. J. A. McGrath, and M. H. G. Munro, *Tetrahedron Letters*, 1966, 3173.

termination of the co-ordinates of 72 independent non-hydrogen atoms in the two molecules for the complete evaluation of the crystal and molecular structure of this compound.

Three dimensional X-ray diffraction data from these crystals were collected by the stationary crystal-stationary counter technique.<sup>11</sup> Corrections were applied for background scattering by use of balanced filter pairs of nickel and cobalt, the intensities being counted for 10 s with each filter. Diffraction data were collected to a Bragg angle of 70° with Cu-K $\alpha$  radiation. The crystals deteriorated on prolonged exposure to X-rays and hence several crystals were used during the entire data collection. Intensities from different crystals were scaled to a common value by having an adequate number of duplicate measurements with each crystal. The diffraction intensity data were converted into appropriate structure amplitudes of the reflection in the usual way, applying geometrical and empirical absorption corrections. A total of 5831 non-zero reflections were measured and used for the structure determination.

*Structure Analysis.*—The asymmetric unit contains four bromine atoms and their positions were established by analysis of the three dimensional sharpened Patterson map. Three of the bromine atoms had very similar  $y$  parameters and the fourth was quite different. These bromine positions were used as a starting point for evaluating the phase angles of the reflections. Electron density maps were computed to establish the rest of the structure in a series of structure factor, least squares, and Fourier cycles. As more atoms were revealed, they were included in the next structure factor cycles for phasing the reflections. At the end of six cycles, the positions of all the 72 non-hydrogen atoms in the two molecules were established. The progress of the refinement is shown in Table 1. The atomic co-ordinates

TABLE 1

Stage	Progress of the refinement *	Value of R
1	Calculation of sharpened Patterson maps to locate the four bromine positions	
2	S.f.c. using four bromine positions, followed by l.s. applying anisotropic temperature factors for bromines and calculations of electron density maps	0.47
3	2 Cycles of l.s. and s.f.c. with four bromine plus 10 possible atoms e.d.s. followed	0.36
4	2 Cycles of l.s. and s.f.c. with the four bromines plus 30 possible atoms e.d.s. followed	0.30
5	2 Cycles of l.s. and s.f.c. with the four bromines plus 41 possible atoms e.d.s. followed	0.28
6	2 Cycles of l.s. and s.f.c. with the four bromines plus 66 possible atoms e.d.s. followed	0.22
7	2 Cycles of l.s. and s.f.c. with the four bromine plus 68 possible atoms e.d.s. followed and carbon and oxygen identified	0.18
8	Using anisotropic temperature for all atoms and using 5190 reflections of $F_o > \sigma$ , 10 more cycles of l.s. and s.f.c. with all atoms	0.10

\* l.s. = Least-squares, s.f.c. = structure factor calculation, e.d.s. = electron density synthesis.

and their standard deviations for the two molecules A and B are given in Table 2. The list of observed

TABLE 2  
Fractional co-ordinates  $\times 10^4$ , with estimated standard deviations in parentheses

Atom	Molecule A		$z/c$
	$x/a$	$y/b$	
Br(A1)	1927(0)	0(2)	-982(1)
Br(A2)	2340(0)	3891(2)	1384(1)
O(12')	1204(3)	1742(28)	442(12)
O(12'')	1500(3)	-1150(23)	948(9)
O(17')	3369(3)	-4731(10)	-738(5)
O(17'')	2776(9)	-3751(53)	-1671(25)
O(19')	4966(1)	-2348(6)	3385(3)
O(19'')	5658(3)	-3731(16)	4128(6)
O(20')	5827(1)	1642(8)	2336(3)
O(20'')	5689(2)	4439(8)	1718(3)
C(1)	3902(2)	1819(9)	1877(4)
C(2)	4388(2)	2829(12)	2395(5)
C(3)	4781(2)	1446(9)	2914(4)
C(4)	4979(2)	42(9)	2283(3)
C(5)	4498(1)	-832(8)	1673(3)
C(6)	4656(2)	-2238(8)	1027(3)
C(7)	4187(2)	-3330(10)	642(4)
C(8)	3717(2)	-2203(9)	180(4)
C(9)	3568(1)	-699(8)	824(3)
C(10)	4060(1)	488(6)	1196(3)
C(11)	3080(2)	441(9)	387(3)
C(12)	2574(2)	-93(10)	750(3)
C(13)	2082(2)	933(17)	332(5)
C(14)	2011(3)	2938(21)	217(9)
C(15)	1416(4)	3270(24)	59(13)
C(16)	1552(4)	343(30)	654(10)
C(17)	3217(3)	-3347(10)	-123(5)
C(17')	3067(4)	-5089(17)	-1396(7)
C(17'')	3244(4)	-6599(16)	-1950(7)
C(18)	4235(2)	1591(8)	431(3)
C(19)	5325(2)	-1383(10)	2843(4)
C(19')	5207(3)	-3480(12)	3961(5)
C(19'')	4816(4)	-4360(13)	4503(5)
C(20)	5374(2)	934(9)	1750(4)
C(20')	5945(2)	3466(10)	2235(5)
C(20'')	6434(3)	3963(13)	2896(7)
Molecule B			
Br(B1)	3228(0)	5614(2)	5088(1)
Br(B2)	2545(0)	10321(2)	3254(0)
O(12')	3668(2)	8015(13)	3543(5)
O(12'')	3269(3)	5865(16)	2709(6)
O(17')	1700(2)	1668(8)	5574(3)
O(17'')	2519(3)	809(16)	5858(5)
O(19')	-187(1)	3745(6)	1588(2)
O(19'')	-820(2)	1668(9)	1316(4)
O(20')	-926(1)	7640(6)	2900(3)
O(20'')	-756(2)	10319(8)	3517(3)
C(1)	969(2)	7929(9)	2862(4)
C(2)	442(2)	8849(9)	2451(4)
C(3)	22(2)	7478(9)	2058(3)
C(4)	-103(2)	6060(8)	2739(3)
C(5)	421(1)	5223(8)	3267(3)
C(6)	338(2)	3833(9)	3978(3)
C(7)	852(2)	2845(9)	4297(3)
C(8)	1321(1)	4043(8)	4625(3)
C(9)	1397(2)	5478(8)	3896(3)
C(10)	884(1)	6602(8)	3618(3)
C(11)	1906(1)	6626(10)	4196(3)
C(12)	2335(2)	6215(12)	3613(3)
C(13)	2869(2)	7131(13)	3913(5)
C(14)	2968(5)	9023(34)	4198(9)
C(15)	3570(4)	9322(25)	4244(3)
C(16)	3284(3)	6812(17)	3291(7)
C(17)	1821(2)	2876(10)	4891(4)
C(17')	2082(2)	780(13)	6007(5)
C(17'')	1924(5)	-524(15)	6667(7)
C(18)	778(2)	7882(9)	4411(3)
C(19)	-455(1)	4599(9)	2277(3)
C(19')	-429(2)	2373(9)	1146(4)
C(19'')	-158(3)	1810(12)	401(5)
C(20)	-431(2)	7009(13)	3409(5)
C(20')	-1048(2)	9407(9)	3044(4)
C(20'')	-1590(3)	9853(12)	2534(6)

<sup>11</sup> T. C. Furnas and D. Harker, *Rev. Sci. Instr.*, 1955, **26**, 449.

and calculated structure factors and the anisotropic thermal parameters for the atoms are deposited as Supplementary Publication No. SUP 21129 (8 pp.).\*

*Description of the Structure.*—Figure 1 is an ORTEP<sup>12</sup> drawing of the dibromowightionolide triacetate molecule as viewed along the *b* axis, using the thermal

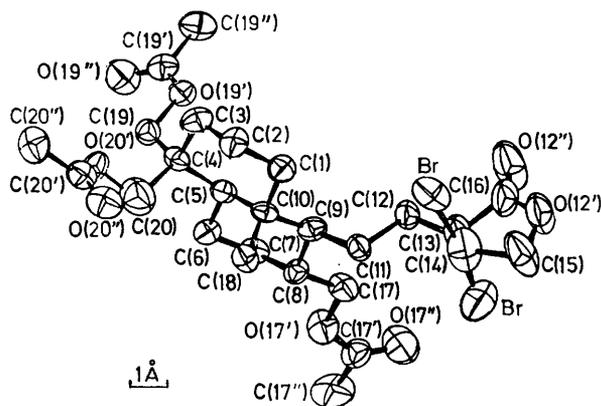


FIGURE 1 ORTEP drawing of dibromowightionolide triacetate

TABLE 3

Interatomic distances (Å) *			
C(1)—C(2)	1.55	C(13)—C(16)	1.57
C(1)—C(10)	1.55	C(13)—Br	2.16
C(2)—C(3)	1.54	C(14)—C(15)	1.53
C(3)—C(4)	1.55	C(14)—Br	1.96
C(4)—C(5)	1.57	C(15)—O(12')	1.45
C(4)—C(19)	1.52	C(16)—O(12'')	1.34
C(4)—C(20)	1.57	C(16)—O(12''')	1.17
C(5)—C(6)	1.54	C(17)—O(17')	1.47
C(5)—C(10)	1.57	C(17'')—O(17'')	1.24
C(6)—C(7)	1.49	C(17''')—C(17''')	1.51
C(7)—C(8)	1.52	C(17''')—O(17''')	1.21
C(8)—C(9)	1.57	C(19)—O(19')	1.50
C(8)—C(17)	1.53	C(19'')—O(19'')	1.30
C(9)—C(10)	1.55	C(19''')—C(19''')	1.52
C(9)—C(11)	1.56	C(19''')—O(19''')	1.17
C(10)—C(18)	1.56	C(20)—O(20')	1.45
C(11)—C(12)	1.55	C(20'')—O(20'')	1.38
C(12)—C(13)	1.52	C(20''')—C(20''')	1.52
C(13)—C(14)	1.47	C(20''')—O(20''')	1.18

\* Average standard deviation estimated as 0.015 Å.

ellipsoids to denote the anisotropic vibration parameters, and showing the atomic numbering. The packing of the four molecules in the unit cell of the crystal is shown in Figure 2. Tables 3 and 4 give the average values of the bond lengths and bond angles of the two molecules in the asymmetric unit.

There are no hydrogen bonds available for stabilizing the crystal structure and the molecules are held together mainly by van der Waals forces. The two six-membered rings are in the chair form. The torsion angles in the rings are given in Table 5, and it is seen that they are significantly less than 60°, and the valency angles are greater than 109.5°, a general phenomenon exhibited by chair-form six-membered rings. Equations for the planes through the fused six-membered rings of molecule A and molecule B are given in Table 6 and the distance of each atom from the plane in Table 7.

\* For details see Notice to Authors No. 7, in *J.C.S. Perkin I*, 1973, Index issue.

TABLE 4

Bond angles (deg.) \*

C(10)—C(1)—C(2)	112	C(14)—C(13)—C(16)	104
C(1)—C(2)—C(3)	112	C(14)—C(13)—Br	102
C(2)—C(3)—C(4)	111	C(13)—C(14)—C(15)	106
C(3)—C(4)—C(5)	111	C(13)—C(14)—Br	102
C(3)—C(4)—C(19)	108	C(15)—C(14)—Br	112
C(5)—C(4)—C(20)	110	C(14)—C(15)—O(12')	102
C(19)—C(4)—C(20)	106	C(13)—C(16)—O(12')	106
C(4)—C(5)—C(6)	115	C(13)—C(16)—O(12'')	125
C(4)—C(5)—C(10)	117	O(12'')—C(16)—O(12''')	128
C(6)—C(5)—C(10)	111	C(15)—O(12'')—C(16)	115
C(5)—C(6)—C(7)	110	C(8)—C(17)—O(17')	106
C(6)—C(7)—C(8)	115	C(17)—O(17')—C(17'')	118
C(7)—C(8)—C(9)	109	O(17'')—C(17'')—O(17''')	123
C(7)—C(8)—C(17)	112	O(17'')—C(17'')—C(17''')	115
C(9)—C(8)—C(17)	110	C(17''')—C(17'')—O(17''')	124
C(8)—C(9)—C(10)	111	C(4)—C(19)—O(19')	108
C(8)—C(9)—C(11)	110	C(19)—O(19')—C(19'')	116
C(10)—C(9)—C(11)	114	O(19'')—C(19'')—O(19''')	125
C(1)—C(10)—C(5)	109	O(19'')—C(19'')—C(19''')	112
C(5)—C(10)—C(9)	108	C(19'')—C(19'')—O(19''')	123
C(1)—C(10)—C(18)	109	C(4)—C(20)—O(20')	108
C(9)—C(10)—C(18)	109	C(20)—O(20')—C(20'')	115
C(9)—C(11)—C(12)	111	O(20'')—C(20'')—O(20''')	122
C(11)—C(12)—C(13)	114	O(20'')—C(20'')—C(20''')	109
C(12)—C(13)—C(16)	114	C(20'')—C(20'')—O(20''')	128
C(12)—C(13)—Br	105		

\* Average standard deviation estimated as 1.5°.

TABLE 5

Torsion angles around the two fused six-membered rings (deg.) (molecule A and molecule B are crystallographically unrelated)

Ring A	Molecule A	Molecule B
C(4)—C(3)—C(2)—C(1)	59.0	57.7
C(3)—C(2)—C(1)—C(10)	—61.8	—60.8
C(2)—C(1)—C(10)—C(5)	53.8	52.9
C(1)—C(10)—C(5)—C(4)	—48.8	—47.6
C(10)—C(5)—C(4)—C(3)	48.6	45.5
C(5)—C(4)—C(3)—C(2)	—51.4	—48.4
Ring B		
C(10)—C(5)—C(6)—C(7)	—59.6	—56.3
C(5)—C(6)—C(7)—C(8)	56.9	55.3
C(6)—C(7)—C(8)—C(9)	—53.6	—54.4
C(7)—C(8)—C(9)—C(10)	54.2	56.1
C(8)—C(9)—C(10)—C(5)	—58.3	—59.5
C(9)—C(10)—C(5)—C(6)	60.6	59.5

Torsion angles in the lactone rings

C(13)—C(16)—O(12')—C(15)	—3.1	—4.7
C(16)—O(12')—C(15)—C(14)	—12.7	—15.2
O(12')—C(15)—C(14)—C(13)	23.8	29.9
C(15)—C(14)—C(13)—C(16)	—24.9	—33.5
C(14)—C(13)—C(16)—O(12')	17.7	24.0

TABLE 6

Equations of the planes for six-membered rings and lactone rings \*

Plane	Equation	Average deviation (Å)
A1	—0.4994X — 0.5222Y + 0.6913Z = —3.56	0.25
B1	—0.4012X + 0.5413Y + 0.7390Z = 5.80	0.24
A2	0.0825X + 0.3229Y + 0.9428Z = 1.27	0.11
B2	0.2757X — 0.5665Y + 0.7766Z = 3.26	0.15

\* X, Y, and Z are orthogonal axes parallel to crystallographic axes *a*, *b*, and *c*.\*

The torsion angles calculated for the lactone rings in the two molecules are also given in Table 5. The

<sup>12</sup> C. K. Johnson, 'A Fortran Thermal-Ellipsoid Plot Program for Crystal Crystal Structure Illustrations,' ORNL 3794, Oak Ridge National Laboratory, 1965.

TABLE 7

Distances (Å) of atoms from the best plane

## Plane A1

C(4) -0.09, C(3) 0.37, C(2) -0.27, C(1) 0.12, C(10) -0.37,  
C(5) 0.13, C(6) -0.30, C(7) 0.26, C(8) -0.12, C(9) 0.26

## Plane B1

C(4) 0.08, C(3) -0.33, C(2) 0.27, C(1) -0.13, C(10) 0.36,  
C(5) -0.14, C(6) 0.27, C(7) -0.25, C(8) 0.14, C(9) -0.26

## Plane A2

C(13) -0.14, C(16) 0.07, O(12') 0.03, C(15) -0.11, C(14)  
0.16

## Plane B2

C(13) 0.18, C(16) -0.09, O(12') -0.03, C(15) 0.14, C(14)  
-0.21

angles show that both lactone rings have a C(14) envelope conformation. Equations for the planes through the lactone rings of molecules A and B are also in Table 6 and the distance of each atom from the plane in Table 7.

Figure 3 contains Newman projections of the conformations about C(1)-C(10), C(10)-C(5), C(10)-C(9), C(14)-C(13), C(4)-C(19), C(4)-C(20), C(8)-C(17), and C(9)-C(11), for both molecules A and B; they all show more or less staggered conformation. Side chain C(4)-C(19) is equatorial and C(4)-C(20) axial to the ring. C(8)-C(17) and C(9)-C(11) appear to be both equatorial to the fused six-membered rings.

The angle formed by planes A1 and A2 is 64° and by planes B1 and B2, 81°. There seem to be no significant stereochemical difference between the two molecules in the asymmetric unit.

## EXPERIMENTAL

U.v. and i.r. spectra were taken on Beckmann DK-2A and Perkin-Elmer Infracord spectrophotometers respectively. N.m.r. spectra were determined with Varian A-60

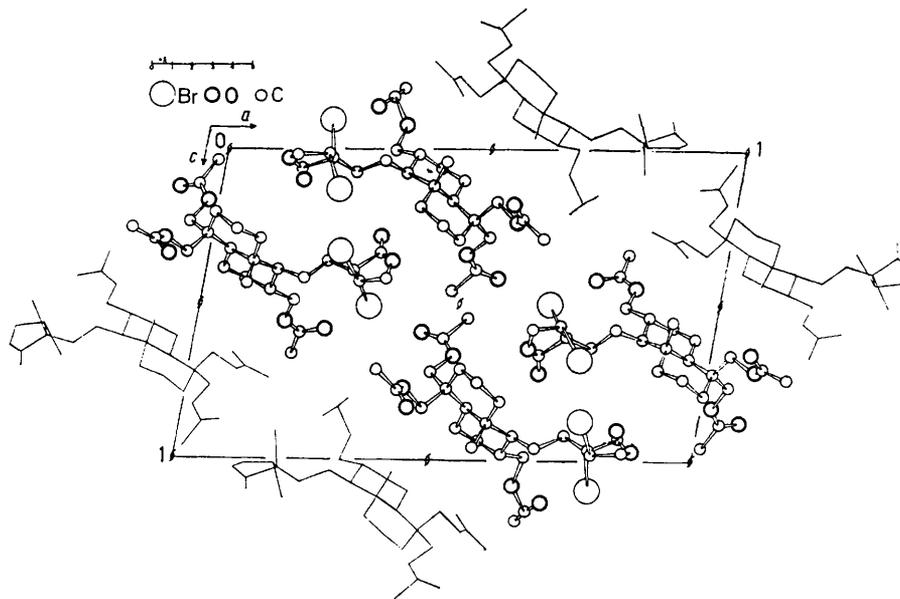
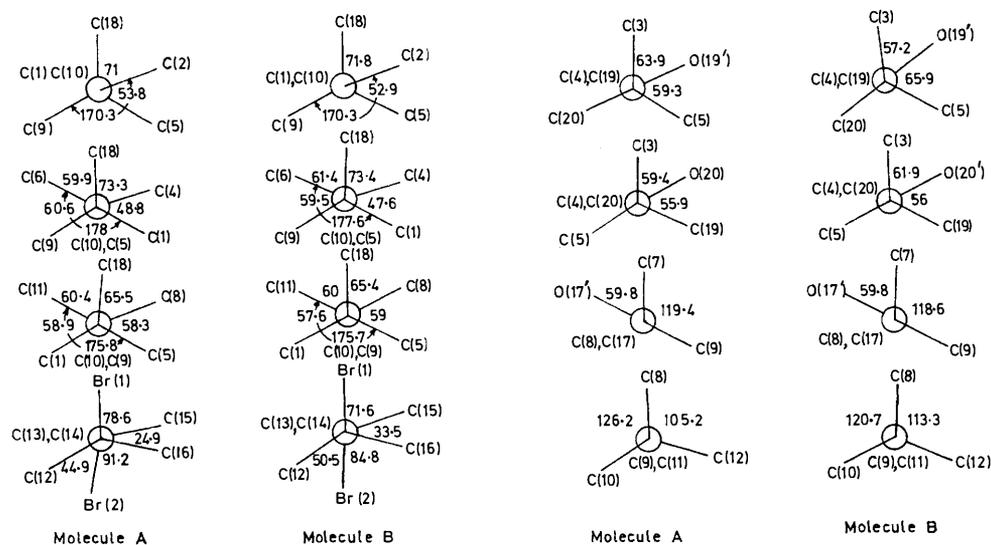
FIGURE 2 Molecular packing in a unit cell projected along the *b* axis

FIGURE 3 Newman representations of conformations of dibromowightionolide triacetate

or HA-100 spectrometers using tetramethylsilane as the internal standard.

*Isolation of Wightionolide (I), Echioidin, and Echioidinin.*—

(a) The dried leaves of *Andrographis wightiana* (20 kg) were extracted with hot chloroform (2 × 50 l) for 2 h. The extracts were combined, concentrated to a thick syrup, diluted with ethyl acetate, and kept overnight. The precipitate was collected and triturated with methanol. The methanol-soluble fraction on evaporation gave a residue (65 g) which crystallized as greenish-white needles from ethyl acetate. This was further purified by passing the methanolic solution through neutral alumina. The crystalline material (30 g) showed three spots on t.l.c. This partially purified mixture (30 g) was dissolved in chloroform–5% methanol and chromatographed on a column of silica gel (0.05–0.2 mm; 900 g). Fractions (100 ml) were collected and monitored by t.l.c.

Fractions 21–50 (chloroform–5% methanol) yielded *wightionolide* (17,19,20-trihydroxy-5 $\beta$ ,8 $\alpha$ H,9 $\beta$ H,10 $\alpha$ -labd-13-en-16,15-olactone) (XII) as plates (from ethyl acetate) (27 g), m.p. 176°,  $R_F$  0.4 (chloroform–7% methanol; silica gel),  $[\alpha]_D -17.5^\circ$  ( $c$  2.085, AcOH),  $\lambda_{max}$  (EtOH) 214 nm ( $\log \epsilon$  3.9),  $\nu_{max}$  (Nujol) 3540, 3300, 3080, 1735, 1695, 1640, 1300, 1255, 1240, 1205, 1165, 1110, 1055, 1045, 1020, 1010, 1000, 980, 950, 940, 930, 920, 870, 850, 835, and 720  $cm^{-1}$ ,  $\delta$  [(CD<sub>3</sub>)<sub>2</sub>SO], 7.38br (1H, s), 4.75br (2H, s), 4.1 (m,  $\geq$ CCH<sub>2</sub>OH), 3.5 (m,  $\geq$ CCH<sub>2</sub>OH), and 0.8 (3H, s,  $\geq$ CMe) (Found: C, 68.1; H, 8.9%;  $M^+$ , 352. C<sub>20</sub>H<sub>32</sub>O<sub>5</sub> requires C, 68.1; H, 9.1%;  $M$ , 352).

(b) The methanol-insoluble residue from (a) was extracted with hot methanol for 16 h. On concentration and cooling the extract gave yellow needles, m.p. 273°, of echioidinin, identical with an authentic sample (mixed m.p., t.l.c., i.r., and acetyl derivative).

(c) After extraction with chloroform, the leaves were further extracted with methanol. An amorphous residue was obtained on concentration. This crystallized from pyridine–methanol to give small white needles, m.p. 293°, of echioidin, identical with an authentic sample (mixed m.p., i.r., and acetyl derivative).

*Dihydrowightionolide* (II).—Wightionolide (500 mg) in ethanol (30 ml) was hydrogenated over 10% palladium-charcoal (250 mg) at room temperature and atmospheric pressure. Removal of the catalyst and evaporation of the solvent gave a gum which crystallized from methylene chloride–ether to afford 17,19,20-trihydroxylabd-16,15-olactone (II) (150 mg), m.p. 167–169°,  $[\alpha]_D -7.6^\circ$  ( $c$  2.44, AcOH),  $\delta$  [(CD<sub>3</sub>)<sub>2</sub>SO] 0.75 (s,  $\geq$ CMe) (Found: C, 67.7; H, 9.6%;  $M^+$ , 354. C<sub>20</sub>H<sub>34</sub>O<sub>5</sub> requires C, 67.7; H, 9.6%;  $M$ , 354).

*Dihydrowightionolide Triacetate.*—Dihydrowightionolide (100 mg) dissolved in pyridine (0.5 ml) was treated with acetic anhydride (1 ml) and the mixture was kept at room temperature overnight. Work-up in the usual way gave an amorphous product which was homogeneous by t.l.c.,  $\delta$  (CCl<sub>4</sub>) 4.1 (m,  $\geq$ CCH<sub>2</sub>OAc), 2.05 (9H, s, AcO), and 0.9 (3H, s,  $\geq$ CMe) (Found: C, 65.1; H, 8.5. C<sub>26</sub>H<sub>40</sub>O<sub>8</sub> requires C, 65.0; H, 8.4%).

*Wightionolide Triacetate* (IV).—Wightionolide (200 mg) was acetylated as usual (Ac<sub>2</sub>O–C<sub>5</sub>H<sub>5</sub>N), to afford the *triacetate* (IV) as silky needles (from ether) (200 mg), m.p. 80°,  $[\alpha]_D -11.7^\circ$  ( $c$  2.06),  $\lambda_{max}$  (EtOH) 210 nm ( $\log \epsilon$  3.98),  $\nu_{max}$  (Nujol) 1735 ( $\alpha\beta$ -unsaturated- $\gamma$ -lactone) and 1720  $cm^{-1}$  (acetate),  $\delta$  (CDCl<sub>3</sub>) 7.1br (1H, s), 4.75 (2H, q,  $J$  1.5 Hz), 4.1 (6H, m,  $\geq$ CCH<sub>2</sub>OAc), 2.03 (3H, s, AcO), 2.04

(6H, s, AcO), and 0.85 (3H, s,  $\geq$ CMe) (Found: C, 65.0; H, 8.1%;  $M^+$ , 478. C<sub>26</sub>H<sub>38</sub>O<sub>8</sub> requires C, 65.2; H, 8.0%;  $M$ , 478).

*Hydrolysis of the Triacetate* (IV).—The acetate (IV) (1.2 g) was dissolved in ethanol (48 ml) containing 2N-sodium hydroxide (32 ml) and kept at room temperature overnight. The ethanol was removed under vacuum and the residue was dissolved in water and acidified with 2N-hydrochloric acid (35 ml). The precipitate gave needles (from ethyl acetate) (340 mg), m.p. 173–175°, identical (t.l.c., i.r. spectrum, and analytical values) with wightionolide.

*Dibromowightionolide Triacetate* (V).—To a solution of (IV) (9.56 g) in chloroform (300 ml) was added calcium carbonate (2 g) and bromine (3.2 g) in chloroform (200 ml). The progress of the reaction was followed by t.l.c. After 48 h additional bromine (1 g) in chloroform (60 ml) was added and the mixture was kept for 5 days. It was then filtered, the solvent was removed, and the residue crystallized from chloroform–hexane. By slow crystallization from aqueous ethanol, 17,19,20-triacetoxy-13,14-dibromolabd-16,15-olactone (V) was obtained as stout rods (3.2 g), m.p. 165–167°,  $R_F$  0.61 (chloroform–ethyl acetate 19:1, silica gel),  $\nu_{max}$  (CH<sub>2</sub>Cl<sub>2</sub>) 2940, 1800, 1740, 1460, 1400, 1370, 1230, 1185, 1170, 1040, 1030, 970, 910, 890, 860, and 820  $cm^{-1}$  (Found: C, 49.3; H, 6.2; Br, 25.2%;  $M^+$ , 638. C<sub>26</sub>H<sub>38</sub>Br<sub>2</sub>O<sub>8</sub> requires C, 48.9; H, 6.0; Br, 25.0%;  $M$ , 638).

*Conversion of the Dibromo-derivative* (V) into (IV).—The dibromo-derivative (V) (200 mg) was heated with zinc dust (1 g) and acetic acid (10 ml) at 60° for 15 h. The mixture was filtered, diluted with water, and extracted with methylene chloride. The organic layer was washed with 10% sodium hydrogen sulphite and then water, and evaporated. The residue crystallized from ether (55 mg), m.p. 80°, and was identical with wightionolide triacetate (mixed m.p., t.l.c., and i.r.).

*Selenium Dehydrogenation of Wightionolide.*—Wightionolide (1 g) and selenium (1 g) were intimately mixed and heated in a sealed tube at 300° for 4 h. The product was extracted with ether and treated with hexane. The precipitate was discarded and the ether–hexane was washed with 5% sodium hydrogen carbonate, 5% sodium hydroxide, and water. The residue obtained after removal of the solvent was taken up in hexane and chromatographed over a short column of alumina. The eluate was distilled at 140° and 0.2 mmHg and its adduct prepared by treating it with *sym*-trinitrobenzene. Recrystallization gave a product (105 mg), m.p. 145–146°, identical with the trinitrobenzene adduct of 1,2,5-trimethylnaphthalene.

*Pyrolysis of Wightionolide.*—An intimate mixture of wightionolide (500 mg) and copper bronze (1 g) was heated in a stream of nitrogen for 10 min at 280°. The gas evolved was collected in 40% methanol, to which was added dione (200 mg) in ethanol (5 ml). The precipitate was identified as the dione derivative of formaldehyde.

*Benzylidene Derivative of Wightionolide.*—A mixture of wightionolide (200 mg), freshly distilled benzaldehyde (8 ml), and fused zinc chloride (200 mg) was kept at room temperature for 15 days. The excess of benzaldehyde was removed by steam-distillation and the mixture was extracted with methylene chloride. Removal of the solvent gave a residue (240 mg) which was purified by chromatography over neutral alumina and then silica gel to give the 19,20-benzylidenedioxy-derivative as an amor-

phous substance (60 mg) homogenous by t.l.c.,  $R_F$  0.73 (chloroform–2% methanol) (Found: C, 73.6; H, 7.8.  $C_{27}H_{38}O_5$  requires C, 73.6; H, 8.2%).

**19,20-Di-*o*-tritylwightionolide.**—Wightionolide (500 mg), trityl chloride (1.25 g), and pyridine (10 ml) were heated at 100° for 2.5 h and then kept at room temperature for 12 h. The mixture was poured into water and extracted with methylene chloride. The organic layer was worked up in the usual manner and the residue taken up in benzene and chromatographed on neutral alumina to give the *diether* as an amorphous residue (170 mg), homogenous t.l.c.,  $\lambda_{max}$  (EtOH) 205 nm (log  $\epsilon$  4.9) (Found: C, 83.0; H, 7.4.  $C_{58}H_{80}O_5$  requires C, 83.2; H, 7.2%).

A solution of the *diether* (418 mg) in ether (30 ml)–ethyl acetate (10 ml) was cooled to 0° and Jones reagent (1.1 ml) added dropwise with stirring during 20 min. The mixture was stirred for a further 1.5 h and diluted with water. The organic layer was separated, washed with 10% sodium hydrogen carbonate, then water, and evaporated. An amorphous product was obtained (350 mg),  $R_F$  0.46 (benzene–chloroform 1:1, silica gel), which gave a positive reaction for an aldehyde group,  $\delta$  (CDCl<sub>3</sub>) 9.75 (s, CHO).

**Acetonide (VI) of Wightionolide.**—A solution of wightionolide (500 mg) and anhydrous copper(II) sulphate (1.5 g) in dry acetone (50 ml) was kept at room temperature for 24 h. The mixture was filtered and the filtrate evaporated *in vacuo*. The oily residue was dissolved in chloroform and chromatographed on silica gel. This gave a fraction,  $R_F$  0.4 (chloroform–5% methanol) which crystallized as needles of 17-hydroxy-19,20-isopropylidenedioxyabd-13-en-16,15-olactone (VI) (from methylene chloride–hexane) (130 mg), m.p. 133°,  $\nu_{max}$  (Nujol) 3540, 1750, 1730, 1260, 1220, 1195, 1150, 1100, 1050, 1010, 925, 855, and 830  $cm^{-1}$ ,  $\delta$  (CDCl<sub>3</sub>) 7.35 (1H), 4.8 (2H), 1.2 (3H, s,  $\geq$ CMe), 1.3 (3H, s,  $\geq$ CMe), and 0.76 (3H, s,  $\geq$ CMe) (Found: C, 70.4; H, 9.3.  $C_{23}H_{36}O_5$  requires C, 70.3; H, 9.2%).

**Oxidation of Wightionolide with Chromic Acid–Pyridine.**—Wightionolide (500 mg) was added to chromium trioxide–pyridine complex (500 mg in 5 ml) and the mixture was stirred at room temperature for 24 h. It was diluted with benzene, filtered, and the filtrate was washed with dilute hydrochloric acid, water, and dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation gave an oil (200 mg) which was chromatographed on silica gel. Elution with chloroform–2% methanol gave a gum (120 mg),  $R_F$  0.75 (chloroform–7% methanol, silica gel),  $\nu_{max}$  (CH<sub>2</sub>Cl<sub>2</sub>) 3680, 3620, 2500 (OH), 1740 (lactone), 1720, and 1700  $cm^{-1}$  (CHO),  $\delta$  (CDCl<sub>3</sub>) 9.8 (1H, s), 9.6 (1H, d), and 9.3 (1H, s, CHO).

**Oxidation of Wightionolide Triacetate (IV) with Potassium Permanganate.**—To a solution of the triacetate (IV) (1.5 g) in acetone (75 ml) at 0°, small portions of potassium permanganate (2.4 g) were added during 2 h till a pink colour persisted. The mixture was evaporated to dryness *in vacuo* and the residue treated with 2*N*-sulphuric acid (30 ml) and extracted with ethyl acetate (4 × 25 ml). The ethyl acetate extract after washing with water gave a residue (1.3 g) which was dissolved in chloroform and chromatographed on silica gel. Elution with chloroform gave

3,7,7-trisacetoxymethyl-1-methylbicyclo[4.4.0]decan-2-ylacetic acid (VII) (600 mg) which crystallized from ether,  $R_F$  0.4 (chloroform–5% methanol, silica gel) (Found: C, 61.9; H, 8.2%;  $M^+$ , 426.  $C_{22}H_{34}O_8$  requires C, 61.9; H, 8.0%;  $M$ , 426). The acid on treatment with ethereal diazomethane gave the *methyl ester* (VIII) (42 mg), m.p. 112–114°,  $R_F$  0.75 (chloroform–7% methanol, silica gel),  $\delta$  (CDCl<sub>3</sub>) 4.1 (6H, m,  $\geq$ CCH<sub>2</sub>OAc), 3.65 (3H, s, CO<sub>2</sub>Me), 2.05 (9H, AcO), and 0.82 (3H, s,  $\geq$ CMe) (Found: C, 62.6; H, 8.4%;  $M^+$ , 440.  $C_{23}H_{36}O_8$  requires C, 62.7; H, 8.2%;  $M$ , 440).

**Oxidation of Wightionolide Triacetate (IV) with Potassium Permanganate–Sodium Metaperiodate.**—A mixture of the triacetate (IV) (2.24 g), potassium carbonate (1.6 g), potassium permanganate (1 g), and sodium metaperiodate (7.05 g) in 3% dioxan–water (800 ml) was stirred at room temperature for 20 h. The mixture was acidified with 2*N*-sulphuric acid, extracted with ethyl acetate, and the organic layer was washed with water. Evaporation of the solvent gave a residue (2.2 g) which was dissolved in chloroform and chromatographed on silica gel. Elution with chloroform–5% methanol gave the 3,7,7-trisacetoxymethyl-1-methylbicyclo[4.4.0]decan-2-ylpropionic acid (IX), which crystallized from ether–hexane (1.14 g), m.p. 110–112°,  $R_F$  0.35 (ethyl acetate, silica gel),  $\nu_{max}$  (CH<sub>2</sub>Cl<sub>2</sub>) 3500, 2940, 1740, 1520, 1470, 1440, 1380, 1360, 1285, 1230, 1125, 1110, 1070, 1040, 975, 950, 910, and 855  $cm^{-1}$  (Found: C, 62.8; H, 8.2%;  $M^+$ , 440.  $C_{23}H_{36}O_8$  requires C, 62.7; H, 8.2%;  $M$ , 440). The acid on treatment with ethereal diazomethane gave the *methyl ester* (X), b.p. 200° at 5 × 10<sup>-4</sup> mmHg, m.p. 44°,  $R_F$  0.5 (chloroform, silica gel) (Found: C, 63.3; H, 8.7.  $C_{24}H_{38}O_8$  requires C, 63.4; H, 8.4%).

**Oxidation of Wightionolide (I) with Potassium Permanganate–Sodium Metaperiodate.**—A mixture of wightionolide (2.7 g), dioxan (450 ml), potassium carbonate (2.4 g), potassium permanganate (1.5 g), sodium metaperiodate (10.56 g), and water (750 ml) was stirred at room temperature for 22 h. The mixture was acidified with dilute sulphuric acid, extracted with ethyl acetate, and the organic layer was shaken with 10% sodium hydrogen carbonate and then water. Removal of the solvent gave a gum (900 mg) which crystallized from ethyl acetate–ether to give 8,8-bishydroxymethyl-11a-methylperhydronaphth-[2,1-c]oxepin-3-one (XI) (110 mg), m.p. 164–165°,  $R_F$  0.35 (ethyl acetate, silica gel),  $\nu_{max}$  (CH<sub>2</sub>Cl<sub>2</sub>) 3620, 3500, 2940, 2860, 1735, 1620, 1590, 1500, 1470, 1440, 1400, 1350, 1340, 1310, 1290, 1215, 1165, 1130, 1100, 1080, 1070, 1060, 1040, 1010, 990, 970, 950, 930, 910, 890, 870, and 850  $cm^{-1}$  (Found: C, 68.5; H, 9.5; O, 21.7%;  $M^+$ , 296.  $C_{17}H_{28}O_4$  requires C, 68.9; H, 9.5; O, 21.6%;  $M$ , 296).

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