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An X-ray Diffraction Determination of the Chemical Structure of Colchicine

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X-ray measurements have been made on a number of colchicine derivatives, in a search for a crystal suitable for a structure analysis of the colchicine molecule. The methylene bromide and iodide addition complexes, $C_{22}H_{25}NO_6.CH_2X_2$, are isomorphous, in space-group $P2_12_12_1$, with 4 molecules in a cell of dimensions $a=9{\cdot}11$, $b=14{\cdot}87$, $c=18{\cdot}54$ Å for the bromide, and $a=9{\cdot}22$, $b=15{\cdot}05$, $c=18{\cdot}63$ Å for the iodide. A straightforward analysis, utilizing the isomorphism and the non-negativity criterion for phase determination on X-RAC, led to a-axis projections which confirmed the ring structure proposed by Dewar, involving one six- and two seven-membered rings. The substituents are in positions corresponding to those suggested by Čech & Šantavy for colchiceine. The rather flat colchicine molecules lie one above the other in columns, and chains of hydrogen bonds link the columns together in pairs. The methylene halide molecules are held in discrete cages between the columns.

Introduction

A study of the crystal structure of colchicine was undertaken because of recent interest in the ring system of this biologically active compound, and uncertainty in position of atoms and groups attached to the rings (Loudon, 1948, 1951). The chief problem in this investigation was the preparation of derivatives suitable for X-ray analysis.

Selection of suitable crystalline derivatives

Colchicine crystallizes as a dihydrate in a unit cell, with

$$a = 13{\cdot}0,\quad b = 10{\cdot}6,\quad c = 16{\cdot}2\ \text{Å}\,,$$
$$\alpha = 90°,\quad \beta = 110°,\quad \gamma = 90°\,.$$

There are four molecules of $C_{22}H_{25}NO_6.2H_2O$ in the cell. There are indications that the space group is $P2_1$, but the exact symmetry was not determined.

It seemed worthwhile to look for molecular compounds or other derivatives of the alkaloid, crystals of which showed more favorable features for a structure analysis: e.g., a short axis, along which a projection is centro-symmetric; a heavy atom derivative; or, preferably, an isomorphous series of derivatives, the members of which differ in containing atoms of significantly different scattering powers. The following crystals were examined.

Crystals of the molecular compound with $HAuCl_4$ were prepared according to the method of Zeisel (1886) and recrystallized from ethyl acetate. These were found to have the orthorhombic space group $P2_12_12_1$ or $P2_12_12$, with four molecules in a cell of dimensions $a = 12{\cdot}2$, $b = 21{\cdot}9$, $c = 10{\cdot}1$ Å. The only apparent disadvantage of these crystals was the excessively high absorption and scattering power of the gold atoms, which would have required very carefully measured and absorption-corrected intensities.

Crystals containing chloroform of crystallization, as first reported by Merck (1916), were found to be twinned, as were the crystals of the molecular compound with $CHBrCl_2$; these compounds are almost certainly isomorphous. Both compounds lose solvent of crystallization rapidly on exposure to the atmosphere, but X-ray photographs showed that the chloroform compound could be preserved for a day or two by enclosure in a gelatin capsule.

A molecular compound with methyl iodide was found to be orthorhombic with space group $P2_12_12_1$ or $P2_12_12$, $a = 22$, $b = 34$, $c = 14$ Å, with probably 16 molecules of colchicine per unit cell. Like the chloroform compound, this derivative decomposes rapidly on exposure to the atmosphere, and further study was not attempted.

$RhCl_3$ gives a gelatinous precipitate with colchicine from methanol solution. Attempts to crystallize this precipitate did not succeed.

Crystals of N-acetyliodocolchinol were supplied by Dr G. Ullyot of Smith, Kline and French. These universally showed complex twinning, which could not be eliminated through recrystallization under various conditions.

It was found that complexes could be formed with methylene bromide and methylene iodide, and that these were stable and could be recrystallized in advantageous size from acetone. These were isomorphous, had favorable cell dimensions and space group, and contained intact molecules of colchicine. The structure analysis was consequently carried through on these.

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Table 1. *Final position for* $B.CH_2I_2$ *in* $P2_12_12_1$

	Y	Z		Y	Z		Y	Z
I_1	0·42	0·25	C_1	0·56	0·31	C_{13}	0·63	0·57
I_2	0·21	0·28	C_2	0·61	0·33	C_{14}	0·56	0·62
			C_3	0·63	0·39	C_{15}	0·48	0·60
N	0·32	0·47	C_4	0·61	0·45	C_{16}	0·42	0·56
			C_5	0·56	0·43	C_{17}	0·63	0·18
O_1	0·63	0·27	C_6	0·54	0·37	C_{18}	0·64	0·38
O_2	0·69	0·41	C_7	0·49	0·35	C_{19}	0·55	0·55
O_3	0·64	0·51	C_8	0·40	0·37	C_{20}	0·27	0·48
O_4	0·41	0·63	C_9	0·38	0·45	C_{21}	0·28	0·53
O_5	0·60	0·67	C_{10}	0·45	0·49	C_{22}	0·69	0·68
O_6	0·23	0·46	C_{11}	0·53	0·49	C_{23}	0·28	0·24
			C_{12}	0·60	0·52			

Methylene halide complexes

The methylene halide complexes have formulae $C_{22}H_{25}NO_6.CH_2X_2$, are orthorhombic with space group $P2_12_12_1$, and have lattice parameters

$$B.CH_2Br_2:\quad a=9{\cdot}11\pm0{\cdot}01,\ b=14{\cdot}87\pm0{\cdot}01,\ c=18{\cdot}54\pm0{\cdot}01\ \text{Å};$$
$$B.CH_2I_2:\quad a=9{\cdot}22\pm0{\cdot}01,\ b=15{\cdot}01\pm0{\cdot}01,\ c=18{\cdot}63\pm0{\cdot}01\ \text{Å}.$$

A measured density (floating method) of 1·71 g.cm.$^{-3}$ for the $B.CH_2I_2$ led to four molecules in the unit cell. Optical examination showed the crystals to be biaxial, negative.

Diffraction photographs of the derivatives were taken on a Buerger precession camera with Mo $K\alpha$ radiation, using approximately spherically ground crystals. Intensities were measured visually by comparison with intensity standards. The intensities were corrected for Lorentz polarization factors using Buerger's formula (Buerger, 1944).*

Patterson maps projected along the a and b axes were prepared for both derivatives on X-RAC, the electronic analogue computer for X-ray analysis (Pepinsky, 1947). From these maps the coordinates of the halogen atoms were roughly determined. The halogen contributions to the F_{0kl}'s were calculated, and these values were used to put the observed structure factors of the two derivatives on the same scale. Comparison of the differences between observed F's with the differences between calculated contributions of halogen atoms determined the signs of a majority of the $(0kl)$ terms. Electron-density summations projected along the a axis were made with these trial signs for both derivatives on X-RAC. In these summations, the origin was displaced $\frac{1}{4}$ in the y direction from that used in the *International Tables*; this permits the use of cosine terms only.

The map of $B.CH_2I_2$ showed fairly good resolution, permitting a tentative orientation of the molecule in the unit cell. With these positions, structure factors were calculated which gave another set of signs. The same carbon, nitrogen and oxygen positions were used in the preparation of the map for $B.CH_2Br_2$. New Fourier maps on $(0kl)$ showed some improvement in resolution and peak heights, but indicated changes in the position of some atoms. This process was repeated six times, for both compounds, and after the last Fourier a consistent set of signs was achieved. The positions of the light atoms are not exactly the same in both compounds. In the later stages of this approximation the testing of signs on X-RAC, using the principle of non-negativity of the background (Eiland & Pepinsky, 1950) proved very useful. These final maps are given in Fig. 1 and 3, as tracings of X-RAC patterns with molecules superimposed in the asymmetric unit. The deduced structure of the colchicine

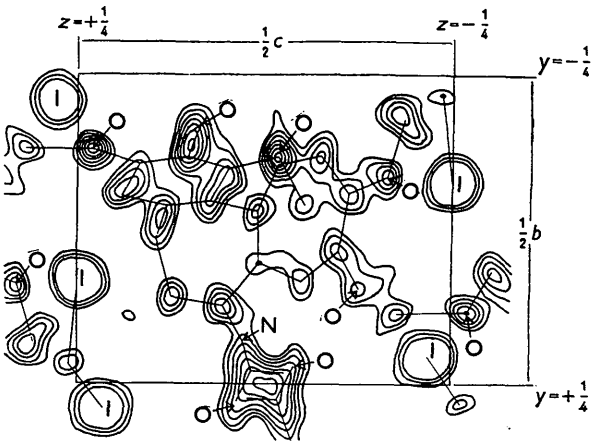

Fig. 1. Electron-density map of colchicine.CH_2I_2, projected along a.

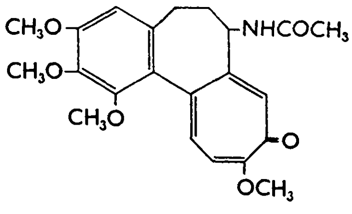

Fig. 2. Proposed structure of colchicine.

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* J. Waser in a paper 'The Lorentz Factor for the Buerger Precession Method' presented at the Washington meeting of the A. C. A., 15 February 1951, proved that this expression is somewhat in error. The error is, however, quite small for the values of the precession angle used in this study.

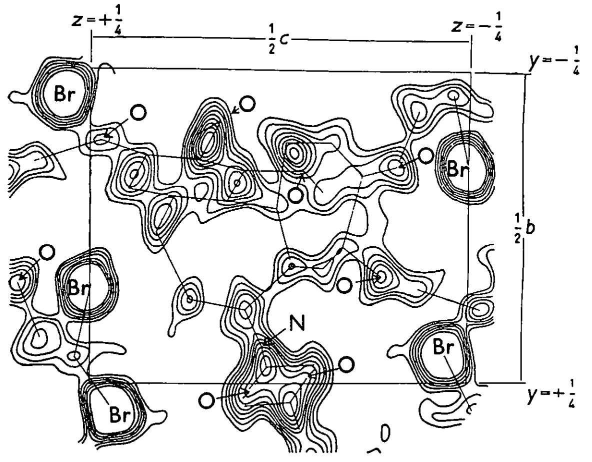


Fig. 3. Electron-density map of colchicine . CH_2Br_2 projected along *a*.

molecule is shown in Fig. 2. Atomic coordinates are given in Table 1.

Agreement between observed and calculated scattering

The observed structure factors were brought onto an absolute scale by comparing them in groups of the same $\sin\theta$ with the calculated factors, and drawing a correction curve against $\sin\theta$. The comparison of observed and calculated amplitudes is presented in Table 2.

The reliability factor $R = \Sigma|F_o-F_c| \div |\Sigma|F_o|$ (not including the very few non-observed reflections) was found for each:

$$R = 21\cdot1\,\%, \quad \text{for} \quad B.CH_2I_2;$$
$$R = 32\cdot3\,\%, \quad \text{for} \quad B.CH_2Br_2\,.$$

Principle of the structure

The structure confirms the ring structure proposed by Dewar (1945) and the positions of the substituents as suggested by Čech & Šantavy (1949).

The principle of packing is seen to be that of infinite columns of nearly flat colchicine molecules

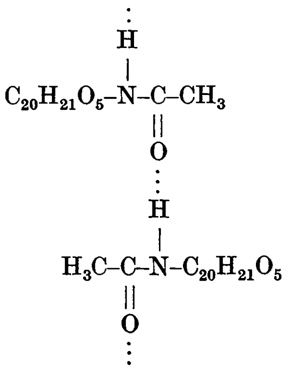
Table 2. *Comparison between observed and calculated structure factors for* $B.CH_2I_2$

hkl	F_c	F_o	*hkl*	F_c	F_o	*hkl*	F_c	F_o
002	−213	180	0,2,14	113	76	051	52	83
004	344	270	0,2,15	126	106	052	40	80
006	−215	184	0,2,16	−201	166	053	28	36
008	204	183	0,2,17	−162	132	054	−113	125
0,0,10	−133	137				055	30	40
0,0,12	49	79	031	145	195	056	176	178
0,0,14	− 50	74	032	31	29	057	− 16	22
0,0,16	29	51	033	− 36	22	058	−178	216
			034	−162	204	059	65	106
011	24	21	035	164	143	0,5,10	142	115
012	4	12	036	130	149	0,5,11	− 22	30
013	− 2	14	037	−109	127	0,5,12	−108	120
014	− 21	22	038	− 51	26	0,5,13	31	39
015	154	192	039	92	108	0,5,14	40	24
016	42	26	0,3,10	82	66	0,5,15	− 34	70
017	−119	90	0,3,11	− 85	38	0,5,16	− 71	69
018	− 26	15	0,3,12	− 85	58			
019	121	110	0,3,13	52	72	060	223	260
0,1,10	21	29	0,3,14	59	55	061	128	147
0,1,11	−183	182	0,3,15	− 64	45	062	−133	137
0,1,12	− 14	—	0,3,16	20	90	063	− 67	44
0,1,13	40	39	0,3,17	− 2	30	064	172	166
0,1,14	14	23				065	96	109
0,1,15	− 43	37	040	−112	185	066	−134	126
0,1,16	− 45	45	041	298	240	067	− 7	—
0,1,17	− 37	35	042	27	24	068	131	129
			043	−275	283	069	37	43
020	15	24	044	17	12	0,6,10	−171	169
021	1	16	045	248	224	0,6,11	63	58
022	− 46	74	046	34	100	0,6,12	109	100
023	92	65	047	−232	242	0,6,13	−104	88
024	111	145	048	− 53	32	0,6,14	−139	102
025	−114	124	049	131	116	0,6,15	167	148
026	4	24	0,4,10	84	102	0,6,16	138	161
027	50	58	0,4,11	− 68	100			
028	− 87	88	0,4,12	−100	105	071	14	21
029	−177	209	0,4,13	49	64	072	89	152
0,2,10	100	127	0,4,14	95	115	073	21	—
0,2,11	163	131	0,4,15	− 7	25	074	− 97	118
0,2,12	−143	137	0,4,16	−161	130	075	− 74	37
0,2,13	−188	151	0,4,17	− 37	46	076	110	97

Table 2 (*cont.*)

hkl	F_c	F_o
077	44	37
078	−105	122
079	− 45	34
0,7,10	163	137
0,7,11	27	32
0,7,12	−137	131
0,7,13	− 58	35
0,7,14	45	42
0,7,15	60	73
0,7,16	− 77	132
080	−140	157
081	− 14	38
082	157	183
083	36	—
084	−139	117
085	− 30	18
086	133	126
087	109	140
088	− 72	73
089	−110	128
0,8,10	33	61
0,8,11	131	107
0,8,12	− 28	35
0,8,13	−198	159
0,8,14	20	34
0,8,15	142	105
091	− 41	19
092	50	20
093	26	32
094	− 52	48
095	− 83	73
096	115	125
097	66	76
098	− 61	82
099	−147	129
0,9,10	79	65
0,9,11	132	147
0,9,12	− 48	35
0,9,13	− 67	87
0,9,14	61	81
0,10,0	−204	159
0,10,1	199	186
0,10,2	178	193
0,10,3	−179	200
0,10,4	−112	98
0,10,5	142	128
0,10,6	83	86
0,10,7	−127	162
0,10,8	− 44	42
0,10,9	127	84
0,10,10	47	84
0,10,11	− 46	61
0,10,12	4	41
0,10,13	100	87
0,11,1	− 11	—
0,11,2	− 2	—
0,11,3	79	68
0,11,4	15	—
0,11,5	− 76	42
0,11,6	41	50
0,11,7	116	153
0,11,8	− 15	36
0,11,9	−122	115
0,11,10	15	34
0,11,11	110	94
0,12,0	− 81	110
0,12,1	− 34	—
0,12,2	− 25	44
0,12,3	25	—
0,12,4	0	43
0,12,5	− 49	36
0,12,6	− 47	50
0,12,7	59	82
0,12,8	92	45
0,12,9	8	40
0,12,10	−152	136
0,13,1	0	—
0,13,2	0	37
0,13,3	23	26
0,13,4	56	45
0,13,5	−127	95
0,13,6	− 24	42
0,13,7	88	80
0,14,0	−147	144
0,14,1	−164	143
0,14,2	168	171
0,14,3	180	158
0,14,4	−171	153
0,14,5	− 93	54
0,14,6	120	107
0,14,7	131	104

resting on one another, two columns being linked together by a chain of hydrogen bonds running in the *a* direction, thus:



$$\begin{array}{c} \vdots \\ \mathrm{H} \\ | \\ \mathrm{C_{20}H_{21}O_5{-}N{-}C{-}CH_3} \\ \| \\ \mathrm{O} \\ \vdots \\ \mathrm{H} \\ | \\ \mathrm{H_3C{-}C{-}N{-}C_{20}H_{21}O_5} \\ \| \\ \mathrm{O} \\ \vdots \end{array}$$

The molecules of methylene halide are held in interstices between the columns, in discrete cages rather than in continuous channels. Thus the molecules of CH_2X_2 cannot escape freely, which explains why the molecular compound is stable on exposure to air.

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