The Crystal and Molecular Structure of the 1:1 Complex of Acetone with the Lactoid Form of Fluorescein

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Single crystals of the 1:1 complex of the lactoid form of fluorescein with acetone are irregular yellow monoclinic lumps, with $a = 12.4014$ (5), $b = 15.8012$ (10), $c = 9.5875$ (8) Å, $\beta = 95.394$ (5)°, $P2_1/n$, $Z = 4$. The crystal structure, based on 2933 intensities measured on a diffractometer, was derived by direct methods and refined to R 0.046. The C–O bond in the lactone ring, which ruptures to give the fluorescent form of the molecule, is 1.525 (3) Å. The acetone is hydrogen-bonded (2.739 Å) to a hydroxyl group and lies in a large cavity. Another weaker intermolecular hydrogen bond (2.891 Å) occurs between the other hydroxyl group and a lactone carbonyl oxygen.

Introduction

Fluorescein has been well known and widely used for many years in the form either of a microcrystalline red powder, or of an amorphous yellow powder. Single crystals up to a few mm in dimensions were first obtained in 1967 by Dr D. H. Leaback from acetone, and their infrared spectrum indicated the presence of acetone and suggested that it was somehow bound to the fluorescein. As the crystals fluoresced the molecules were thought to be in the carboxylic form, and an X-ray study was begun in the hope that it might be possible eventually to correlate the plane of polarization of the fluorescent radiation from a single crystal with the molecular orientation, and so hopefully make it possible to infer the orientation of fluorescein molecules complexed on to the surfaces of proteins or nucleotides. The crystal was, however, shown to contain lactoid molecules (I) and a re-examination showed that the fluorescence was emanating from a virtually invisible surface film of acetone-free powder.

(I)   (IV)

(II)   (III)
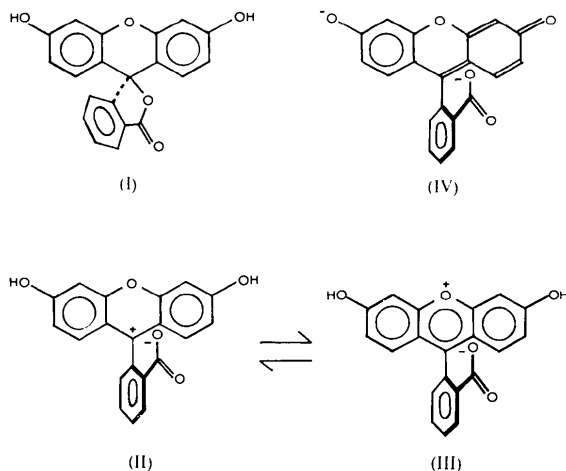
For lack of suitable direct-methods programs, work was shelved in 1967, but resumed later when programs

became available. A fresh batch of crystals was then needed, and was kindly supplied by Dr Leaback.

Experimental

The crystals were clear amber-yellow irregular lumps. Some had one or two plane faces, but none had good overall morphology, and none of the extinction directions were parallel to any observed edges. Crystallographic data: $C_{20}H_{12}O_5 \cdot (CH_3)_2O$; $M = 378.38$, monoclinic; $a = 12.4014$ (5), $b = 15.8012$ (10), $c = 9.5875$ (8) Å, $\beta = 95.394$ (5)°, $V = 1870.4$ Å³; $D_c = 1.344$ g cm⁻³ for $Z = 4$; $F(000) = 792$; $\mu(Cu K\alpha) = 8.5$ cm⁻¹. The non-standard space group $P2_1/n$ (deduced uniquely from systematic absences) was preferred as the β angle for $P2_1/n$ was so much nearer 90° than for the alternatives.

Intensities were collected to a maximum θ of 65° on a Siemens automatic single-crystal diffractometer (AED) by techniques described elsewhere (Allen, Rogers & Troughton, 1971).

The crystal, which had no well defined morphology and maximum dimensions 0.47 mm, was used unencapsulated as the loss of acetone appeared from the early photographic work to be slow. Both the unvarying appearance of the crystal and the constancy of the reference reflexion showed that deterioration was negligible. 3204 unique reflexions were measured of which 271 had $I < 2.58\sigma(I)$ and were classified as unobserved (Allen *et al.*, 1971). The 2933 observed reflexions were corrected for Lorentz and polarization factors and counting drifts (Allen *et al.*, 1971), but no absorption correction was thought necessary. A Patterson map was computed but, as expected, gave no immediately useful information.

The 171 terms with $|E| \geq 2.0$ were used as the basis of $\sum_2$ calculations using Dr M. G. B. Drew's program *PHASEM* (Drew, Templeton & Zalkin, 1969). The initial starting set comprised 3 terms to define the origin and 3 algebraic variable phases a, b, c. In addition the phases of two ggg reflexions were deduced from $\sum_1$ relations of rather weak probability. Two sep-

arate runs of the program *PHISUM* (Drew *et al.*, 1969) were carried out with and without the two *ggg* terms. In numerous instances alternative alphanumeric sums for a phase gave indications that $b - c = a$, and it proved possible to re-express all phases consistently in terms of b only. Comparison of the results for the two runs suggested strongly that $b = \pi$, and substitution of this value gave consistent phases for 116 terms. As a check, hand calculations on a $\sum_2$ listing produced by a program written by a colleague, D. J. Williams, was also carried out and corroborated the phases of about 100 terms.

An E map based on the 116 terms contained a feature (see Fig. 1) representing five contiguous near-coplanar rings in a position and orientation that were consistent with prominent features of the Patterson map. It was reminiscent of the 'stuttering' found in the E maps of several crystals containing regular planar molecules, the spurious peaks apparently being due to lack of the cancelling effects of the numerous weaker reflexions. As there were no clues indicating which three adjacent rings were the real ones, trials were made and each was refined with *ORFLS* (Stewart, 1970). The trio of rings a, b, c (Fig. 1) was the best tried and Fourier and difference maps based on them revealed the whole of the rest of the molecule quite sharply defined. With all nonhydrogen atoms correctly assigned and isotropically refined, R fell to 0·133.

The hydrogen atoms were located from difference syntheses, and the positions of all except the hydroxyl and acetone hydrogens were checked against positions calculated by *BONDLA* (Stewart, 1970), good agreement being obtained. The hydrogen atoms were allowed to refine isotropically in all subsequent cycles of *BLOKLS* (Stewart, 1970), and when all other atoms were refined anisotropically R fell to 0·062. The two reflexions 202 and 10$\bar{1}$ were suspected of extinction and deleted from the data. Adjustments were made to some of the hydrogen parameters because temperature factors of several hydrogens were tending to become negative, and the positions of H(2), H(12), H(16), H(18), H(101) and H(103) were drifting rather close to the atoms to which they were bound. After these adjustments 8 cycles of *BLOKLS* were run with the hydrogens refining isotropically, and all other atoms anisotropically; this brought R down to 0·046. At this stage it was thought advisable to attempt to refine the occupancy of the acetone positions in order to ascertain whether the crystals had deteriorated significantly before the intensity measurements had started. All the acetone atoms were given initial arbitrary occupancies of 0·8, but after four cycles of *BLOKLS*, refining all parameters in the same mode as in the previous run in addition to refining occupancies, the latter all converged to 1·0. It seemed, therefore, that the crystal used had its full quota of acetone throughout the intensity measurements.

The hydrogen parameters mentioned above were again 'drifting' in the same way as before, but all the

non-hydrogen parameters had ceased to alter significantly, except for those of C(34), one of the acetone methyl carbons, whose shifts indicated that refinement was not quite complete. However, since this atom was relatively unimportant and there was not sufficient jus-

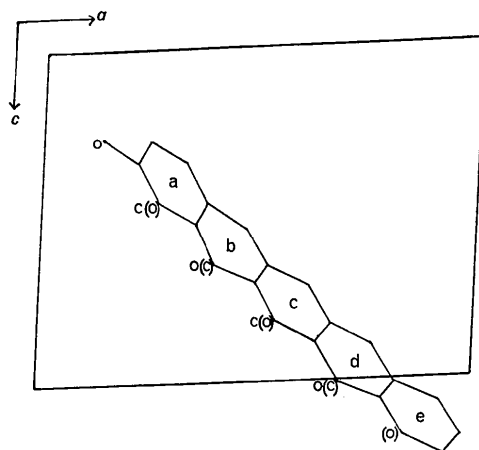Fig. 1. Projection of the pattern of peaks in the E map of fluorescein. Letters in parentheses show the designations of the atoms used in the first trial refinement.
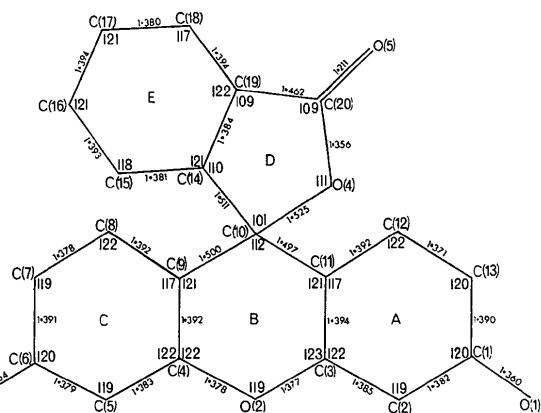

Fig. 2. Bond lengths (Å) and some of the valence angles (°) of fluorescein. A full list of valence angles appears in Table 5.
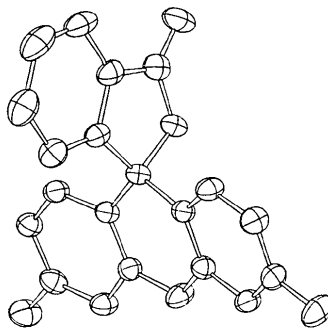

Fig. 3. The fluorescein molecule viewed along **a***. The thermal motions are represented by 50% equiprobability surfaces.

tification for using more computer time, refinement was discontinued.

The 'drifting' of the hydrogen parameters may have been due to the omission of an absorption correction ($\mu = 8\cdot5$ cm^{-1}); the irregularity of the crystal surfaces ruled out any possibility of using the program *ICABS* (Troughton, 1968). The use of the X-RAY System program *DATRDN* (Stewart, 1970) to apply a rough correction (by assuming a mean spherical radius) was also dismissed since even the most pessimistic estimate of the variations of the radius gave only negligible varia-

Table 1. *Fractional atomic coordinates* ($\times 10^5$) *with standard deviations in parentheses*

	x	y	z
C(1)	21227 (17)	47039 (13)	33315 (23)
C(2)	26596 (17)	49545 (13)	45927 (23)
C(3)	34760 (17)	44460 (13)	52219 (21)
C(4)	48795 (16)	43409 (13)	70698 (21)
C(5)	54125 (17)	47428 (13)	82162 (23)
C(6)	63384 (18)	43821 (14)	88718 (22)
C(7)	67317 (18)	36250 (15)	83803 (24)
C(8)	61850 (17)	32402 (14)	72325 (24)
C(9)	52412 (16)	35788 (13)	65536 (21)
C(10)	46057 (16)	31229 (12)	53745 (21)
C(11)	37676 (16)	36840 (12)	46271 (21)
C(12)	32082 (17)	34559 (13)	33557 (22)
C(13)	24034 (18)	39513 (14)	27072 (23)
C(14)	52984 (16)	26320 (13)	44429 (22)
C(15)	60238 (19)	29312 (16)	35516 (25)
C(16)	66042 (21)	23378 (19)	28508 (28)
C(17)	64551 (22)	14723 (19)	30399 (30)
C(18)	57163 (21)	11719 (15)	39099 (27)
C(19)	51420 (17)	17718 (13)	46074 (22)
C(20)	43023 (17)	16445 (13)	55602 (23)
C(32)	42471 (20)	20073 (16)	3068 (25)
C(33)	42401 (25)	29147 (19)	-1161 (32)
C(34)	49136 (31)	14148 (23)	-4701 (35)
O(1)	13148 (13)	51678 (10)	26451 (18)
O(2)	39670 (12)	47467 (9)	64721 (15)
O(3)	68438 (13)	47955 (11)	99987 (17)
O(4)	39942 (11)	24093 (9)	60262 (15)
O(5)	38857 (14)	9988 (10)	59287 (18)
O(6)	37374 (14)	17634 (11)	12478 (18)

Table 2. *Fractional atomic coordinates* ($\times 10^3$) *and isotropic temperature factors* ($\times 10^2$) *for the hydrogen atoms with standard deviations in parentheses*

	x	y	z	B
H(2)	252 (2)	551 (1)	503 (2)	67 (49)
H(5)	515 (2)	525 (1)	852 (2)	64 (48)
H(7)	741 (2)	340 (1)	883 (3)	235 (63)
H(8)	650 (2)	277 (1)	687 (2)	130 (53)
H(12)	341 (2)	299 (1)	298 (2)	92 (52)
H(13)	206 (2)	381 (1)	183 (2)	50 (48)
H(15)	611 (2)	351 (1)	346 (2)	106 (51)
H(16)	717 (2)	250 (2)	211 (3)	381 (79)
H(17)	695 (2)	104 (2)	255 (3)	337 (73)
H(18)	563 (2)	59 (2)	403 (3)	170 (56)
H(101)	129 (2)	562 (2)	305 (3)	204 (61)
H(103)	743 (2)	457 (2)	1018 (3)	251 (65)
H(331)	489 (3)	310 (2)	11 (3)	484 (81)
H(332)	361 (3)	320 (2)	14 (3)	450 (81)
H(333)	406 (3)	294 (2)	-125 (4)	579 (95)
H(341)	567 (3)	164 (2)	-35 (4)	638 (102)
H(342)	453 (3)	137 (2)	-128 (3)	506 (88)
H(343)	498 (3)	87 (2)	-12 (3)	406 (78)

Table 3. *Anisotropic thermal parameters* ($\times 10^5$) *with standard deviations in parentheses*

The anisotropic thermal ellipsoid has the form $\exp[-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}hl + 2\beta_{23}kl)]$.

	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
C(1)	386 (14)	231 (9)	845 (27)	4 (9)	-52 (15)	77 (12)
C(2)	440 (15)	199 (8)	846 (26)	44 (9)	-25 (16)	-26 (12)
C(3)	405 (14)	202 (8)	637 (23)	3 (9)	-10 (14)	-21 (11)
C(4)	366 (14)	207 (8)	658 (24)	22 (9)	21 (14)	14 (11)
C(5)	473 (15)	209 (9)	728 (25)	23 (9)	18 (15)	-72 (12)
C(6)	453 (15)	266 (9)	683 (24)	-38 (10)	-11 (15)	-56 (12)
C(7)	404 (15)	304 (10)	874 (28)	54 (10)	-105 (16)	-42 (13)
C(8)	435 (15)	218 (9)	881 (27)	58 (9)	-20 (16)	-66 (12)
C(9)	378 (14)	185 (8)	612 (23)	0 (8)	16 (14)	5 (11)
C(10)	379 (14)	160 (8)	694 (24)	-9 (8)	45 (14)	6 (11)
C(11)	367 (14)	174 (8)	658 (23)	0 (8)	16 (14)	11 (11)
C(12)	459 (15)	192 (8)	743 (25)	-4 (9)	5 (15)	-48 (12)
C(13)	482 (15)	232 (9)	749 (26)	-27 (9)	-91 (16)	-6 (12)
C(14)	357 (14)	223 (9)	686 (24)	40 (9)	-72 (14)	-71 (12)
C(15)	468 (16)	343 (11)	914 (29)	-5 (11)	85 (17)	-72 (14)
C(16)	537 (18)	559 (15)	1077 (34)	66 (14)	138 (20)	-167 (18)
C(17)	630 (20)	503 (14)	1285 (37)	196 (14)	21 (21)	-348 (19)
C(18)	630 (19)	267 (10)	1162 (33)	126 (11)	-211 (20)	-204 (15)
C(19)	426 (14)	212 (9)	768 (26)	47 (9)	-167 (15)	-80 (12)
C(20)	455 (15)	193 (8)	798 (26)	12 (9)	-253 (16)	1 (11)
C(32)	569 (18)	343 (11)	804 (28)	57 (11)	-24 (18)	-60 (14)
C(33)	917 (25)	406 (13)	1347 (40)	45 (15)	-275 (25)	171 (19)
C(34)	1267 (34)	575 (18)	1471 (47)	207 (20)	382 (32)	-99 (23)
O(1)	537 (12)	289 (7)	1156 (22)	105 (7)	-296 (13)	2 (10)
O(2)	540 (11)	255 (6)	805 (18)	140 (7)	-165 (11)	-134 (9)
O(3)	561 (12)	429 (8)	990 (21)	81 (8)	-231 (13)	-263 (11)
O(4)	441 (10)	184 (6)	831 (18)	-23 (6)	40 (11)	35 (8)
O(5)	677 (13)	212 (6)	1216 (23)	-84 (7)	-199 (13)	105 (10)
O(6)	753 (14)	311 (7)	1122 (22)	-108 (9)	277 (14)	-21 (10)

tions in the values of the correction factor. However, although absorption could have had no significant effect on the main structure refinement, it might nonetheless have been sufficient to influence the relatively sensitive hydrogen parameters.

Analysis of the ΔF's showed that, as expected (Allen *et al.*, 1971), no weighting scheme was needed. A difference map was calculated, based on the coordinates from the last cycle of *BLOKLS* preceding the attempt to refine occupancies, at R 0·046. It showed no electron-density variations greater than $\pm 0·2$ e Å^{-3}. The final average value of the shift/error ratio was about 0·1 for the coordinates of the non-hydrogen atoms in the fluorescein molecule.

Discussion of the structure

The fractional atomic coordinates and anisotropic thermal parameters are listed in Tables 1, 2 and 3.* Bond lengths and angles, which are shown diagrammatically in Fig. 2, are listed in Tables 4 and 5, and the results of several best-planes calculations are given in Table 6.

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* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30688 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Table 4. *Bond lengths* (Å)

For rings *A*, *B* and *C* corresponding bond lengths are shown in pairs.

Rings *A*, *B* and *C* of the fluorescein molecule

O(1)—C(1)	1·360 (3)	1·364 (3)	O(3)–C(6)
C(1)—C(2)	1·382 (3)	1·379 (3)	C(5)–C(6)
C(2)—C(3)	1·385 (3)	1·383 (3)	C(4)–C(5)
C(3)—C(11)	1·394 (3)	1·392 (3)	C(4)–C(9)
C(3)—O(2)	1·377 (3)	1·378 (3)	C(4)–O(2)
C(1)—C(13)	1·390 (3)	1·391 (3)	C(6)–C(7)
C(12)–C(13)	1·371 (3)	1·378 (3)	C(7)–C(8)
C(12)–C(11)	1·392 (3)	1·392 (3)	C(8)–C(9)
C(11)–C(10)	1·497 (3)	1·500 (3)	C(9)–C(10)

Remainder of the fluorescein molecule

C(10)–O(4)	1·525 (3)	H(101)–O(1)	0·83 (3)
O(4)–C(20)	1·356 (3)	H(103)–O(3)	0·81 (3)
C(20)–O(5)	1·211 (3)	H(2)——C(2)	1·00 (2)
C(20)–C(19)	1·462 (3)	H(5)——C(5)	0·92 (2)
C(19)–C(14)	1·384 (3)	H(7)——C(7)	0·98 (3)
C(14)–C(10)	1·511 (3)	H(8)——C(8)	0·92 (3)
C(14)–C(15)	1·381 (3)	H(12)–C(12)	0·85 (2)
C(15)–C(16)	1·393 (4)	H(13)–C(13)	0·94 (2)
C(16)–C(17)	1·394 (4)	H(15)–C(15)	0·94 (2)
C(17)–C(18)	1·380 (4)	H(16)–C(16)	1·08 (3)
C(18)–C(19)	1·394 (3)	H(17)–C(17)	1·06 (3)
		H(18)——C(18)	0·93 (3)

Acetone

C(32)——O(6)	1·212 (3)	H(333)–C(33)	1·09 (4)
C(32)——C(33)	1·490 (4)	H(341)–C(34)	1·00 (4)
C(32)——C(34)	1·493 (5)	H(342)–C(34)	0·87 (3)
H(331)–C(33)	0·88 (3)	H(343)–C(34)	0·93 (3)
H(332)–C(33)	0·96 (3)		

Fig. 3 is a diagram of the fluorescein molecule (omitting hydrogen) computed with *ORTEP* (Johnson, 1965). The molecule is clearly in the lactoid form with the lactone–benzene group nearly perpendicular to the rest of the molecule. Fig. 4 is a *b* axis projection of the packing arrangement with the acetone molecules. This diagram also shows the parallelism of corresponding parts of neighbouring fluorescein molecules, and shows the intermolecular hydrogen bonds.

The standard deviations in Table 1 represent an average uncertainty of 0·0021 Å in the positions of C(1)–

Table 5. *Valence angles* (°)

The standard deviation for all angles is $\sim 0·2°$.

Fluorescein

C(13)–C(1)–C(2)	120·1	C(11)–C(10)–O(4)	106·5
O(1)—C(1)–C(2)	122·8	O(4)–C(10)–C(14)	101·2
O(1)—C(1)–C(13)	117·1	C(10)–C(11)–C(3)	120·7
C(1)—C(2)–C(3)	119·1	C(10)–C(11)–C(12)	122·1
C(2)—C(3)–C(11)	122·0	C(3)—C(11)–C(12)	117·0
C(2)—C(3)–O(2)	115·1	C(11)–C(12)–C(13)	122·1
C(11)-C(3)–O(2)	122·9	C(12)–C(13)–C(1)	119·6
C(3)—O(2)–C(4)	118·7	C(15)–C(14)–C(19)	120·9
O(2)–C(4)–C(9)	122·3	C(10)–C(14)–C(19)	110·1
C(9)—C(4)–C(5)	122·3	C(10)–C(14)–C(15)	129·0
O(2)—C(4)–C(5)	115·4	C(14)–C(15)–C(16)	117·7
C(4)—C(5)–C(6)	119·2	C(15)–C(16)–C(17)	121·1
C(5)—C(6)–O(3)	117·6	C(16)–C(17)–C(18)	121·3
C(5)—C(6)–C(7)	120·3	C(17)–C(18)–C(19)	117·0
O(3)—C(6)—C(7)	122·1	C(14)–C(19)–C(20)	108·8
C(6)—C(7)—C(8)	119·2	C(18)–C(19)–C(14)	122·0
C(7)—C(8)—C(9)	122·2	C(18)–C(19)–C(20)	129·2
C(8)—C(9)—C(4)	116·8	C(19)–C(20)–O(5)	130·3
C(8)—C(9)—C(10)	121·8	O(5)—C(20)–O(4)	120·9
C(4)—C(9)—C(10)	121·3	C(19)–C(20)–O(4)	108·8
C(9)—C(10)–C(14)	113·9	C(20)–O(4)—C(10)	110·9
C(9)—C(10)–C(11)	111·6		

Acetone

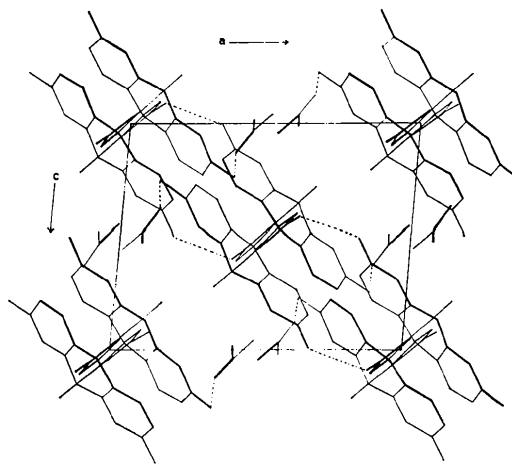| O(6)–C(32)–C(33) | 121·2 | C(33)–C(32)–C(34) | 117·2 |
| O(6)–C(32)–C(34) | 121·6 | | |

Fig. 4. Projection down **b** of the structure of the 1:1 fluorescein–acetone adduct, showing the formation of hydrogen-bonded layers and their mode of packing. Hydrogen bonds are shown as broken lines.

C(14) and C(19), C(20); 0·0026 Å for C(15)–C(18); 0·0030 Å for the acetone carbons C(32)–C(34); and
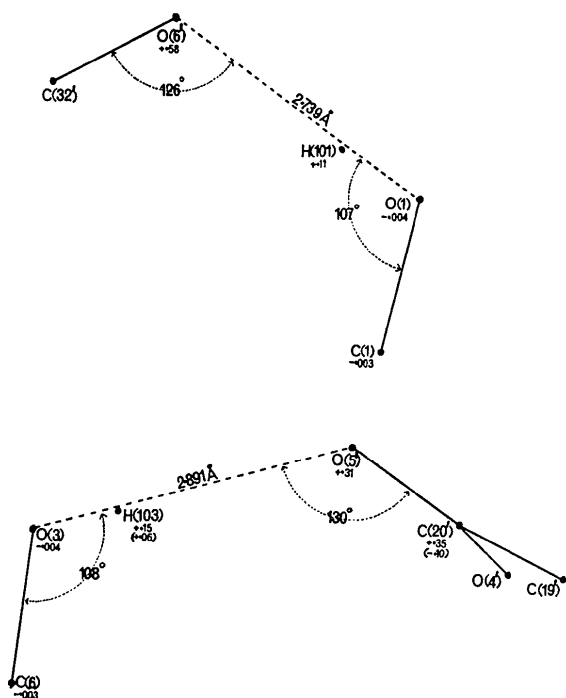

Fig. 5. Plots showing the two hydrogen bonds of fluorescein. Small numbers show the distances of the atoms from the plane of ring A in the upper, and from the plane of ring C in the lower diagram. The corresponding figures in parentheses in the lower diagram are distances from the plane C(6), O(3), O(5′).

0·0016 Å for the oxygen atoms. However, since these figures are based on standard deviations calculated by the block-diagonal refinement program *BLOKLS*, they are doubtless somewhat underestimated. The lower precision obtained for C(15)–C(18) is consistent with the fact that the thermal vibrations of these peripheral atoms of the lactone–benzene group are a little larger than the average vibrations of carbon atoms in the rest of the molecule, the motions being particularly marked in the plane of the lactone–benzene group. This is shown in Fig. 3, from which it appears that the whole of the lactone–benzene group is vibrating in its own plane about the spiro atom C(10), the amplitude of this 'nodding' motion being roughly proportional to the radial distance from C(10). The relatively greater uncertainty in the position for this part of the molecule, and for acetone, is not surprising in view of the large voids surrounding them.

Corresponding bond lengths in the aromatic rings A and C agree well; in fact there is marked symmetry about the line C(10)–O(2) although it is not required by the crystallographic environment. In ring B the bonds (i) C(9)–C(10) and (ii) C(11)–C(10) agree well with each other and are typical of C–C sp^2–sp^3 bonds. In ring D the distance C(14)–C(10), although longer than (i) or (ii), is not abnormal for this type of bond, but the distance C(10)–O(4) [1·525 (3) Å] is exceptionally long and indicates severe weakness in the bond. Such weakness is consistent with the behaviour of fluorescein in solution, where very ready cleavage of C(10)–O(4) yields the fluorescent zwitterion isomer. A C–O bond of 1·48 Å has recently been noted in a six-membered lactone ring in the structure of (±)-dehy-

Table 6. *Least-squares planes and deviations* (*in* Å × 10³)

Atoms used in calculating the planes are marked with an asterisk.
Atoms marked with a prime lie in adjacent molecules.

Plane of ring A

C(1)*	−3;	C(2)*	−1;	C(3)*	4;	C(11)*	−3;	C(12)*	−1	
C(10)	−81;	O(2)	8;	O(1)	−4;	H(101)	107;	O(6′)	585	

Plane of ring C

C(4)*	3;	C(5)*	2;	C(6)*	−3;	C(7)*	−1;	C(8)*	5;	C(9)*	−7
C(10)	−105;	O(2)	17;	O(3)	−4;	H(103)	147;	O(5′)	315;	C(20′)	346

Plane of ring E

C(14)*	8;	C(15)*	−5;	C(16)*	−3;	C(17)*	8;	C(18)*	−4;	C(19)*	−4
C(10)	71;	C(20)	−42;	O(2)	282;	O(4)	17;	O(5)	−128		
C(1)	−3300;	C(2)	−2027;	C(3)	−947;	O(1)	−4410				
C(6)	3794;	C(5)	2610;	C(4)	1417;	O(3)	4951				
C(13)	−3487;	C(12)	−2404;	C(11)	−1106						
C(7)	3786;	C(8)	2582;	C(9)	1367						

Acetone

O(6)*	−1;	C(32)*	2;	C(33)*	−1;	C(34)*	−1
C(1)	1104;	O(1)	−143;	H(1)	−55		

Hydrogen bond

C(6)*	0;	O(3)*	0;	O(5′)*	0		
H(103)	60;	C(20′)	−97;	C(19′)	935;	O(4′)	−1305

Angles between planes

A–C 9·4°; A–E 84·6°; C–E 86·1°

droaltenusin (Rogers, Williams & Thomas, 1971) and, although very much less strained, it has been held responsible for the ready cleavage that occurs during reduction with $Na_2S_2O_4$ to form altenusin.

Table 6 shows that rings A and C, which are inclined at 9·4°, each have a high degree of planarity. O(2) is also close to the planes of A and C, and since the angle C(3)–O(2)–C(4) is 118°, and the bond lengths C(3)–O(2) (1·377 Å) and C(4)–O(2) (1·378 Å) are typical of C–O sp^2–sp^2 bonds, O(2) is probably in the sp^2 state, thus providing a conjugation bridge between rings A and C. However, C(10) deviates significantly from rings A and C to the same side as O(4). C(10) and O(2) both lie to the same side of the plane through C(3), C(4), C(9), C(11), with rings A and C both lying on the opposite side of this plane. Rupture of C(10)–O(4) in fluorescein introduces sp^2 hybridization at C(10) and leads to two tautomeric zwitterions (II), (III), for the red form of the molecule, and this when basified is converted to the fluorescent ion (IV). In forms (II) and (III) rings A, B, C will move slightly to become co-planar and the benzocarboxylic moiety will still be constrained to lie more or less perpendicular to the plane of rings A, B, C. The separation between O(4)⁻ and the positive charge is ~2·7 Å in (II) and ~3·8 Å in (III), but regeneration of the lactoid form is obviously easy in either case.

Each fluorescein molecule appears to participate in two hydrogen bonds; one between the O(1) hydroxyl oxygen and the oxygen of an acetone molecule O(6′) via H(101), and one between the O(3) hydroxyl oxygen and the carbonyl oxygen O(5′) of the glide-related fluorescein molecule via H(103). The existence of these bonds was indicated by the shortness of the O(1)–O(6′) and O(3)–O(5′) distances (see Table 7). Further evidence, which appeared as a by-product of the best-plane calculations, is shown in Fig. 5. In each case, the hydrogen atom is very close to the line joining the oxygen atoms. Table 6 shows that O(1) is close to the plane of the acetone, and since the angle H(101)–O(6′)–C(32′) is close to 120° it appears that the axis of the hydrogen bond O(1)–H(101)–O(6′) lies along one of the lone pairs on O(6′). The hydrogen bond from O(3) to O(5′), however, seems to be in a most unusual direction, viz. between the π and lone-pair orbitals of O(5′), as the

angle H(103)–O(5′)–C(20′) is 130°, and the plane of C(20′)–O(4′)–C(19′) is roughly perpendicular to the plane of C(6)–O(3)–O(5′) (see Fig. 5). These bonds were investigated in some detail as the stability of the lattice was one of the main points of interest in this structure. The acetone is relatively weakly bound by a single hydrogen bond to the fluorescein molecule, but it occupies a large hole in the structure and its main function seems to be the filling of this space to prevent collapse. This may be especially significant during the nucleation of crystals of this particular form, especially as the fluorescein molecule is awkwardly shaped for packing. As mentioned earlier the acetone slowly escapes from the structure, and after 6–8 weeks a surface coating of the dark red powdery form becomes visible.

Table 7. *Shorter intermolecular distances* (Å)

Standard deviations for these distances are only estimates.

O(3)——O(5′)	2·891 (2)
O(1)——O(6′)	2·739 (2)
H(103)–O(5′)	2·08 (3)
H(101)–O(6′)	1·92 (3)

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