The Structure of Fusicoccin A

Culture filtrates of the fungus *Fusicoccum amygdali* Del., the almond shoot canker pathogen, show phytotoxic activity on almond shoots and young tomato cuttings[1]. A crystalline metabolite called fusicoccin A was isolated from submerged cultures grown in stirred fermenters and reported to be a glucoside with the molecular formula $C_{38}H_{58}O_{13}$[2]. Very low concentrations of this metabolite produce symptoms on the leaves of young almond trees which are similar to those which follow infection with the pathogen under natural conditions[3].

The present report describes work which permits one to assign structure I to fusicoccin A. This result was achieved by a restricted use of conventional organic chemistry operations, by an extensive application of NMR and mass spectroscopy, and also by a three-dimensional X-ray analysis of a heavy-atom derivative of I.

The previously reported formula ($C_{38}H_{58}O_{13}$, m.w. 722), which was inferred[2] from the occurrence in the mass spectrum of I of a small peak at *m/e* 704 (considered to be due to M^+-18) and from an X-ray determination of a value of 733.5 ± 14.7 for the molecular weight of I as crystallized from ethyl acetate, must therefore be amended as $C_{36}H_{56}O_{12}$, m.w. 680[4].

General properties of I and its derivatives. Compound I: $C_{36}H_{56}O_{12}$, m.p. 155–156°, $\lambda_{max} < 215$ nm, ν_{max} 3400–3450 (OH), 1725–1740 and 1240 (acetate), 1635 (olefinic), and 920 cm^{-1} (vinyl). The NMR-spectrum shows signals for a vinyl group attached to a quaternary carbon, an olefinic proton on a trisubstituted double bond, an O-Me[6], 2 O-Ac, 2 secondary and 3 tertiary C-Me groups. The mass spectrum shows the molecular peak at *m/e* 680 [minute peaks due to thermal transacetylation ions are observed at *m/e* 722 ($M^+ + 42$), 704 ($M^+ + 42-18$), 689 ($M^+ + 42-18-15$)]; at lower mass values the first prominent peak is at *m/e* 408 (aglycone, $C_{23}H_{36}O_6$ by high resolution mass spectroscopy) and very strong signals are present at *m/e* 205 (monoacetylglucosyl), 69 ($C_5H_9^+$), and 43 (CH_3CO^+).

Dihydro-I, $C_{36}H_{58}O_{12}$, m.p. 151–153°, was obtained by catalytic hydrogenation of I at room temperature. IR- and NMR-spectra indicate that the vinyl group has been reduced and that the trisubstituted double bond is still present. The mass spectrum shows the molecular peak at *m/e* 682, a series of small peaks at higher *m/e* values due to thermal transacetylation, the peak of the aglycone at *m/e* 408, and very strong signals at *m/e* 390 (408–18), 205 (monoacetylglucosyl), 71 ($C_5H_{11}^+$) and 43 (CH_3CO^+). Several ions in the spectrum of both I and dihydro-I can be derived from other ions at *m/e* values + 68 and + 70 respectively, + 60 and + 204; loss of acetic acid is also indicated by metastable peaks.

Triacetyl-I (compound II), $C_{42}H_{62}O_{15}$, m.p. 106–108°, ν_{max} 3500 cm^{-1} (OH), was prepared by room temperature acetylation of I with Ac_2O in dry pyridine. The NMR-spectrum shows 5 acetyl resonances. The mass spectrum shows the molecular peak at *m/e* 806, a peak at *m/e* 450 (monoacetylaglycone, $C_{25}H_{38}O_7$ by high resolution mass spectroscopy), and very strong peaks at *m/e* 289 (triacetylglucosyl), 229 (289–60), 69 ($C_5H_9^+$) and 43 (CH_3CO^+). Metastable peaks for the loss of acetic acid from the triacetylglucose moiety are also present. The peak at *m/e* 289, which shifts to 295 when I is acetylated with $(CD_3CO)_2O$, indicates that the glucosyl moiety in I has one alcoholic function which is not available for acetylation.

Compound III, $C_{39}H_{56}O_{16}$, m.p. 75–76°, ν_{max} 3600 (OH) and 1660 cm^{-1} (olefinic), was obtained in small amount by hydrogenolysis of I followed by acetylation at room temperature, or better still by mild acid hydrolysis of I followed by acetylation. Comparison of its NMR-spectrum with that of II shows that the vinyl group and 2 tertiary C-Me groups are no longer present in III, and that a sixth acetyl group has been introduced. The mass spectrum shows the molecular peak at *m/e* 780, a strong peak at *m/e* 450 (monoacetylaglycone), and very strong peaks at *m/e* 331 (tetraacetylglucosyl) and 43 (CH_3CO^+); the signal at *m/e* 69 is of very low relative intensity compared with that observed in both I and II. Since the glucosyl moiety is fully acetylated in III, the C_5H_9 group, which from the above spectroscopic evidence is clearly a *t*-pentenyl, can be placed on one of the alcoholic functions of the glucose moiety.

Acid hydrolysis of I. Acid hydrolysis of I (0.2*N* HCl in aqueous methanol, 40 h at reflux) yields acetic acid, D-glucose, 2-methylbut-3-en-2-ol and compound IV ($C_{21}H_{34}O_5$). Conclusive evidence for the occurrence of an *O*-*t*-pentenyl group in I was obtained from the identification, by gas-solid chromatography, of *t*-pentyl alcohol and 2-methyl-1-butene in the acid hydrolysate of dihydro-I. The following reaction can therefore be written for the hydrolysis of fusicoccin A: $C_{36}H_{56}O_{12} + 4H_2O = 2CH_3COOH + C_6H_{12}O_6 + CH_2{:}CHC(CH_3)_2OH + C_{21}H_{34}O_5$.

Structural features of deacetylaglycone IV. Compound IV: $C_{21}H_{34}O_5$, m.p. 158–160°, M^+ 366, $\lambda_{max} < 215$ nm, ν_{max} 1635 cm^{-1} (olefinic), and no carbonyl and vinyl bands. Tetra-*O*-acetyl-IV (compound V), $C_{29}H_{42}O_9$, m.p. 110 to 111°, was obtained by room temperature acetylation of IV in dry pyridine with Ac_2O. Its mass spectrum shows the molecular peak at *m/e* 534 and a more pronounced signal at *m/e* 474 ($M^+ - 60$, $C_{27}H_{38}O_7$ by high resolution mass spectroscopy). Detailed consideration of NMR- and NMDR-spectra[7] of IV and V in various solvents led to the following identification of the oxygen substituents in IV: O–CH_3, *s* (3H), not displaced by acetylation: δ^c 3.32, δ^p 3.23 in IV, and δ^c 3.34, δ^p 3.24, δ^b 3.13, δ^{cs} 3.21 in V; the methoxyl is placed on a CH–CH_2 which appears as a

[1] A. Graniti, Phytopath. Medit. *1*, 182 (1962).

[2] A. Ballio, E. B. Chain, P. de Leo, B. F. Erlanger, M. Mauri and A. Tonolo, Nature *203*, 297 (1964).

[3] A. Graniti, Phytopath. Medit. *3*, 125 (1964).

[4] Lately, new mass spectra of I have shown a small peak at *m/e* 722 which is due to thermal transacetylation in the ion source (operated at 200°). Furthermore, it has been firmly established by NMR spectroscopy that crystals of I obtained from ethyl acetate and used in a new X-ray determination[5] of the molecular weight (found: 728 ± 12), contain half a molecule of solvent per molecule of I (m.w. for $C_{36}H_{56}O_{12}$, $^1/_2CH_3CO_2C_2H_5$: 724).

[5] Crystal data for fusicoccin A are: $2C_{36}H_{56}O_{12}$, $CH_3CO_2C_2H_5$, F.W. = 1449.79, monoclinic, $a = 20.78 \pm 0.12$, $b = 14.46 \pm 0.09$, $c = 13.40 \pm 0.06$ Å, $\beta = 95°50' \pm 15'$, $V = 4005$ Å^3, $D_m = 1.207 \pm 0.001$ gcm^{-3} (by flotation), F(000) = 1568. Space group $P2_1$ (C_2^2, No. 4) or $P2_1/m$ (C_{2h}^2, No. 11) from systematic absences. $Z = 2$, $D_x = 1.20 \pm 0.02$ gcm^{-3}. CuKα-radiation (λ taken as 1.5418 Å), $\mu = 7.4$ cm^{-1}. Data derived from Weissenberg photographs, using an improved version of Christ's method[9]. Limits of error given in the form of maximum error.

[6] This was also confirmed by the incorporation of [^{14}C-methyl]-methionine into I; the label was quantitatively retained in III and IV and completely lost on demethylation.

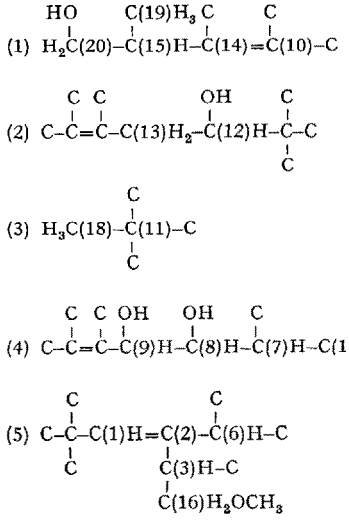
[7] Spectra were recorded on a Varian HA-100 apparatus (internal reference TMS); chemical shifts (accurate within ± 0.02 ppm) are given in δ units, and coupling constants in cps; *s* denotes a singlet, *d* a doublet, *t* a triplet, *q* a quartet, *m* a multiplet, *dd* a doublet of doublets. Depending on the solvent used, the following superscripts are adopted: $c = CDCl_3$, $p = C_5D_5N$, $b = C_6D_6$, $cs = CS_2$.

broadened *d*, or *d* of *m*, *AB* part of an *ABX* system, centred at δ^c 3.32, δ^p3.33 in IV, and at δ^c3.34, δ^p3.37, δ^b 3.29, δ^{cs} 3.23 in V; CH_2OH, a broad unresolved *m* (2H), superimposed on other signals in IV, but shifted to a lower field in V to give the characteristic eight-signal pattern for the *AB* part of an *ABX* system (herein called *A'B'X'*), centred at δ^c 3.98, δ^b 4.07, δ^{cs} 3.82, $J_{A'B'} = 10$; *CH*OH, *m* (1H), centred at $\delta^c \simeq 3.80$, and obscured by other signals in IV, but shifted downfield in V to give a *dd* characteristic of the *X* proton of an *ABX* system (herein called *A"B"X"*), centred at δ^c 4.87, δ^b 5.08, δ^b 5.03, δ^{cs} 4.68: the corresponding *A"B"* protons are found, by means of NMDR, in the range 1.90–2.60 δ^{cs}, giving the characteristic eight-signal pattern with $J_{A''B''} = 16$, and are hence attributed to a methylene joined to a fully substituted carbon; C*H*(OH)C*H*(OH), (2H), corresponding to the *AB* protons of an *ABX* system (herein called *A'''B'''X'''*), $\delta^p_{A'''}$ 4.21, $\delta^p_{B'''}$ 4.43 in IV, and $\delta^p_{A'''}$ 5.51, $\delta^p_{B'''}$ 5.71 in V, $J_{A'''X'''} = 4$, $J_{B'''X'''} < 1$: this α-glycol system was confirmed by the formation of an acetonide ($C_{24}H_{38}O_5$, m.p. 146–147°, 2 new *s*, 3H each, at δ^c 1.35 and 1.84; diacetate, $C_{28}H_{42}O_7$, m.p. 59–60°, M^+ 490) and of a dialdehyde on periodate oxidation.

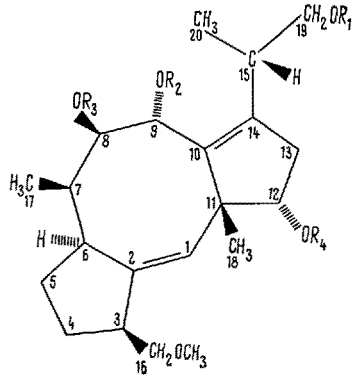
The above assignments not only account for all the oxygen atoms in IV, but also define the nature of 10 out of 12 protons resonating between 3.1 and 6.0 δ in both IV and V. The 2 remaining protons give rise respectively to a *t* (1H, $J = 1.5$, allylic coupling), centred at δ^c 5.38, δ^p 5.90 in IV, and δ^c 5.31, δ^p 5.51 in V (C*H*:C, confirmed by ν_{max} at 1635 cm^{-1}), and to a very complex *m* (1H), best seen in V at δ^{cs} 3.45. Tickling experiments showed that the latter proton is coupled to the methylene of the *A'B'X'* system referred to above.

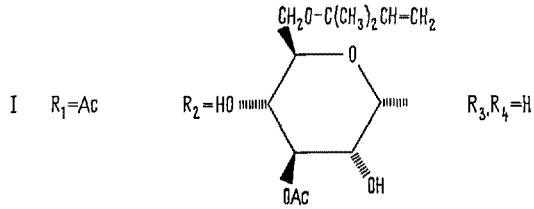
Other features revealed by NMR- and NMDR-spectroscopy consist of: a tertiary C-C*H*$_3$ group (3H, *s*, δ^c 1.20, δ^p 1.34, δ^b 1.33 in IV, δ^c 1.18, δ^p 1.35 in III); a secondary C–C*H*$_3$ group (3H, *d*, δ^c 0.92, δ^p 1.09, $J \simeq 7$ in IV, and δ^c 1.10, δ^p 1.03, $J \simeq 7$ in V), coupled to the above mentioned *X'* proton; another secondary C–C*H*$_3$ group (3H, *d*, δ^c 0.85, δ^p 0.93, $J \simeq 7$ in IV, and δ^c 0.94, δ^p 1.10, $J \simeq 7$ in V), coupled to an unresolved *m* observed in the range 1.9–2.0 δ^c in both IV and V: NMDR also proves that this *m* is the *X'''* part of the *A'''B'''X'''* system; 2 methine protons (2H, two complex overlapping *m* in the range 2.75–3.10 δ^p for both IV and V) split by allylic coupling ($J \simeq 1.5$) with the olefinic proton: one of these methine protons is also coupled to the methylene of the CH_2OCH_3 group; a C*H*$_2$ group adjacent to a quaternary carbon and forming the *A"B"* part of the above quoted *A"B"X"* system, best evidenced in IV as an eight-peak pattern in the range 1.90–2.60 δ^{cs} ($J_{A''B''} = 16$). A tetrasubstituted double bond was indicated by the rather high δ values observed for C(9)*H*, C(13)*H*$_2$ and C(15)*H*.

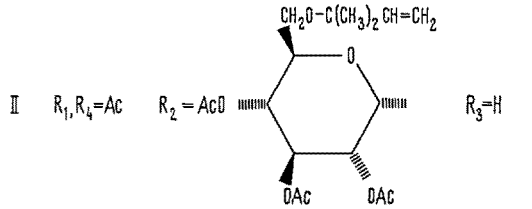
On the above grounds the presence in IV of the partial structures (1–5) was suggested, in which only C(4) and C(5) are not identified:

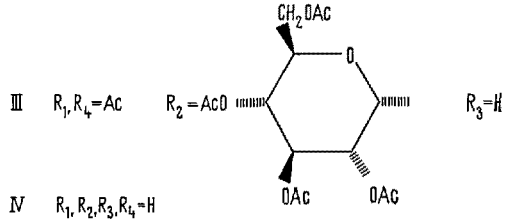


These partial structures, the empirical formula and the observation that radioactivity from [2-^{14}C] mevalolactone is incorporated in vivo in both the *t*-pentenyl group and in the C_{21} moiety of fusicoccin A indicate that IV is the methyl ether of a pentahydroxy-tricarbocyclic terpene. If one is willing to accept that the carbon skeleton of fusicoccin A is derived by cyclization of the geranyl-geranyl cation without subsequent rearrangements, a tentative structure can be derived for IV. This contains the same









IV R_1, R_2, R_3, R_4=H

V R_1, R_2, R_3, R_4=Ac

ring system as the ophiobolins[8], which are also metabolites of plant pathogenic fungi.

Additional support for the presence of one cyclopentane ring in IV was obtained by oxidation of the acetonide of IV with Me_2SO in Ac_2O; the NMR-spectrum of the resulting ketone (λ_{max} 293 nm, ν_{max} 1750 cm^{-1}) showed that C(19)H_2OH had been acetylated and that the CO function arose from the C(12)HOH group of IV (*s*, 2H, δ^c 2.96; C–CH_2CO). This locates the quaternary C(11) between C(10) and C(12).

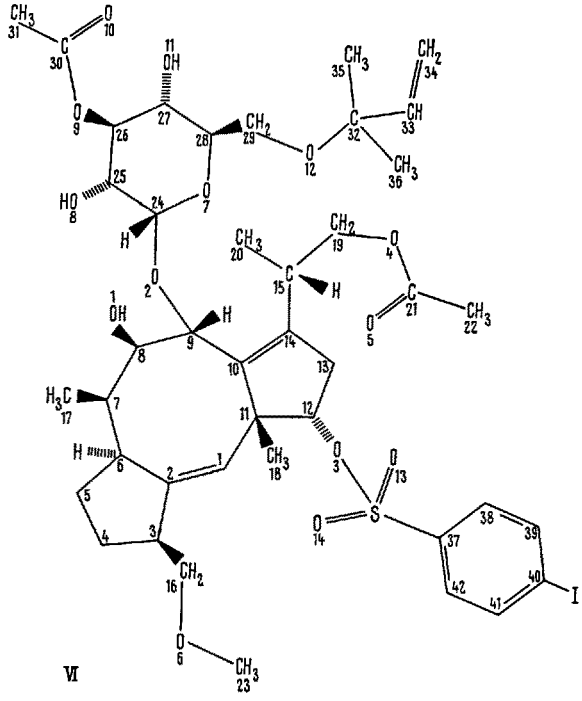
Since strong irradiation of C(7)*H* (δ^c 2.08 in V) decouples one of the 2 allylic protons, C(7) must be linked to either C(3) or C(6). The latter was chosen tentatively on the ground of the isoprene rule; the so far not identified C(4) and C(5) complete then a second cyclopentane ring.

X-ray analysis of a heavy-atom derivative of I. Compound VI, $C_{42}H_{59}IO_{14}S$, m.w. 946, m.p. 148–149°, was prepared by treatment of fusicoccin A with *p*-iodobenzenesulphonyl chloride in dry pyridine at room temperature, purified by column chromatography on Florisil, and crystallized from ethyl acetate.

Crystal data for this compound are: $C_{42}H_{59}IO_{14}S$, F.W. = 946.90, orthorombic disphenoidal, $a = 10.32 \pm 0.06$, $b = 12.89 \pm 0.09$, $c = 35.14 \pm 0.14$ Å, $V = 4674$ Å^3, $D_m = 1.370 \pm 0.003$ gcm^{-3} (by flotation), F(000) = 1968. Space group, $P2_12_12_1$ (D_2^4, No. 19) from systematic absences. $Z = 4$, $D_x = 1.35 \pm 0.023$ gcm^{-3}. CuKα-radiation (λ taken as 1.5418 Å), $\mu = 64.2$ cm^{-1}. Data derived from Weissenberg photographs using an improved version of Christ's method[9]. Limits of error given in the form of maximum error.

The intensity measurements were made by eye on 1706 independent reflections from Weissenberg photographic records taken at room temperature. Absorption corrections were not applied, nor were the extinction corrections.

The iodine atom coordinates were first determined without ambiguity from a three-dimensional Patterson synthesis sharpened on iodine. They corresponded to a *R* value of 40.3%. The structure was then solved completely by a combination of Fourier methods and block-diagonal



VI

Atomic coordinates

Atom	*X/a*	*Y/b*	*Z/c*
C(1)	0.5512	0.2511	0.1312
C(2)	0.5833	0.2267	0.1663
C(3)	0.6181	0.1143	0.1750
C(4)	0.6036	0.1032	0.2183
C(5)	0.6489	0.2151	0.2330
C(6)	0.5856	0.2945	0.2036
C(7)	0.6765	0.3897	0.2055
C(8)	0.6036	0.4888	0.1983
C(9)	0.5521	0.5080	0.1570
C(10)	0.4483	0.4331	0.1457
C(11)	0.4873	0.3514	0.1152
C(12)	0.3366	0.3269	0.1014
C(13)	0.2623	0.3357	0.1370
C(14)	0.3247	0.4182	0.1566
C(15)	0.2636	0.4792	0.1892
C(16)	0.7670	0.0887	0.1601
C(17)	0.8000	0.3804	0.1799
C(18)	0.5666	0.3859	0.0809
C(19)	0.1502	0.5406	0.1699
C(20)	0.2129	0.4072	0.2184
C(21)	0.0156	0.6770	0.1916
C(22)	−0.0420	0.7365	0.2278
C(23)	0.8960	−0.0594	0.1420
C(24)	0.5595	0.6859	0.1302
C(25)	0.5306	0.7967	0.1448
C(26)	0.3814	0.8137	0.1450
C(27)	0.3292	0.7968	0.1077
C(28)	0.3786	0.6819	0.0922
C(29)	0.3005	0.6819	0.0476
C(30)	0.2688	0.9408	0.1776
C(31)	0.2310	1.0588	0.1871
C(32)	0.3048	0.5576	−0.0013
C(33)	0.1856	0.5807	−0.0089
C(34)	0.1551	0.6349	−0.0364
C(35)	0.3577	0.4450	−0.0072
C(36)	0.4283	0.6261	−0.0294
C(37)	0.2777	0.1059	0.0297
C(38)	0.3315	0.1556	0.0007
C(39)	0.3928	0.1001	−0.0329
C(40)	0.3542	−0.0111	−0.0345
C(41)	0.2861	−0.0599	−0.0032
C(42)	0.2474	0.0059	0.0267
O(1)	0.6854	0.5735	0.2110
O(2)	0.5003	0.6181	0.1576
O(3)	0.3396	0.2261	0.0818
O(4)	0.0976	0.6085	0.2021
O(5)	−0.0144	0.6941	0.1571
O(6)	0.7698	−0.0265	0.1619
O(7)	0.5039	0.6707	0.0934
O(8)	0.5872	0.8116	0.1823
O(9)	0.3624	0.9174	0.1558
O(10)	0.1914	0.8774	0.1924
O(11)	0.1931	0.8086	0.1055
O(12)	0.3544	0.5740	0.0364
O(13)	0.1308	0.2578	0.0530
O(14)	0.1477	0.1123	0.0937
S	0.2080	0.1799	0.0667
I	0.4157	−0.0846	−0.0827

[8] K. Tsuda, S. Nozoe, M. Morisaki, K. Hirai, A. Itai, S. Okuda, L. Canonica, A. Fiecchi, M. Galli Kienle and A. Scala, Tetrahedron Lett. 35, 3369 (1967), and previous references quoted therein.

[9] G. Mazzone, A. Vaciago and M. Bonamico, Ric. Sci. *33* (*II-A*), 1113 (1963).

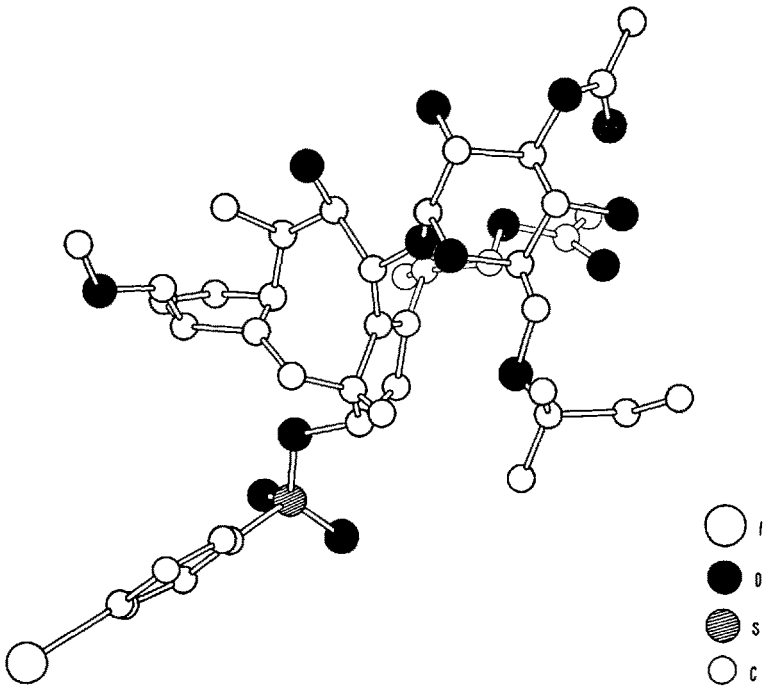


Fig. 1. Molecular model of structure VI as built from the crystallographic parameters.

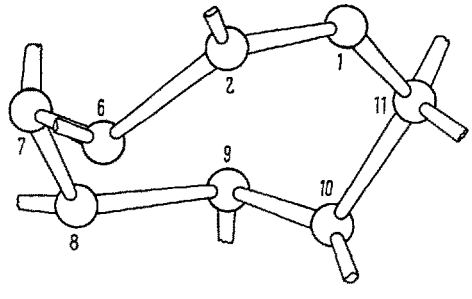


Fig. 2. A view of the eight-membered ring showing the conformation and the numbering scheme.

least-squares calculations in which atoms were judged by the behaviour of their individual isotropic thermal parameters[10].

The final structure is summarized in the Table, where the 58 atoms of compound VI (excluding hydrogens) are listed. The atomic coordinates of the Table correspond, together with the thermal parameters resulting from the refinement[12], to a R value of 11.6%, and define bond lengths which have an average e.s.d. of 0.05 Å and which deviate by a maximum of 0.30 Å (in one case) and by an average of 0.06 Å from acceptable values for formula VI.

The final difference Fourier synthesis ($\sigma(\varrho_0) = 0.2\ e\text{Å}^{-3}$) gave a very smooth residual electron density, oscillating around a nil value with absolute maxima of 0.3–0.4 $e\text{Å}^{-3}$ in the general field (rising to higher values only in the region of the iodine atom). Additional crystallographic evidence taken into account in order to establish structure VI unambigously included: (1) the analysis of the electron population of the Fourier synthesis maxima, (2) stereochemical considerations such as valence angles and the approximate coplanarity of the atoms linked by a double bond and of their substituents, (3) the local analysis of the dependence of the thermal parameters on the choice of either carbon or oxygen for each atom.

Structure VI is fully consistent with the structure proposed for compound IV by chemical and spectroscopic methods, and with the suggested location of the *t*-pentenyl group and of one acetyl group on the glucose moiety. In addition, the X-ray analysis gives the full stereochemistry of the molecule, as well as the exact position of the above substituents and of the glucosyl.

A stereo-model of formula VI is given in Figure 1, showing the configuration of the 13 asymmetric carbon atoms and many other configurational and conformational details, as for instance the fact that the glucose moiety is bonded to the oxygen atom O(2) via an α-glycosidic linkage. The eight-membered ring, seen from a different viewpoint, is also shown in Figure 2.

The absolute configuration is as given in Figure 1: of the 2 mirror images consistent with the X-ray data (no anomalous dispersion measurements were made), the one which corresponds to the known absolute configuration of D-glucose[13] was chosen as the correct absolute configuration of compound VI.

In the ester group at C(26) the oxygen atom O(10) is *cis* to the alcoholic carbon C(26): this is known to be the preferred conformation of the ester group in relation to

[10] All the calculations were carried out on the Rome University IBM 7040 computer, using the SFLS programme of V. ALBANO, A. DOMENICANO and A. VACIAGO[11] and other crystallographic programmes of A. DOMENICANO and A. VACIAGO (unpublished).

[11] V. ALBANO, A. DOMENICANO and A. VACIAGO, Gazz. chim. ital. *96*, 922 (1966).

[12] Isotropic thermal motion was assumed for all atoms, except for iodine and sulphur which were treated anisotropically in the last stages of refinement. Individual isotropic B values range from 2.3 Å² for C(14) to 14.1 Å² for C(34), with an overall average of 5.9 Å². The highest values were found in the *t*-pentenyl and metoxymethyl side chains, and may be due to considerable freedom of movement, or to some positional disorder.

[13] G. N. RAMACHANDRAN, R. CHANDRASEKARAN and K. S. CHANDRASEKARAN, Biochim. biophys. Acta *148*, 317 (1967).

saturated ring systems[14,15]. In the ester group at C(19) as well, the oxygen atom O(5) is *cis* to the alcoholic carbon C(19). The oxygen atom O(2) is in a 'sheltered' position, as a result of the conformation around the C(9)–O(2) and O(2)–C(24) bonds[16].

Riassunto. Con l'uso combinato di metodi chimici, spettroscopici, e di strutturistica chimica diffrattometrica è stato possibile assegnare la struttuta I alla fusicoccina A, metabolita fitopatogeno del fungo *Fusicoccum amygdali* Del.

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Frühe Stadien der Erholung bei bestrahlten Ratten und Mäusen

Mit der sogenannten Split-Dosis-Technik untersucht man häufig den Erholungsverlauf nach Einwirkung ionisierender Strahlen. Das Ausmass der Erholung von einer Erstbestrahlung wird dabei getestet an dem Schaden, den eine zweite Bestrahlung verursacht. Das Kriterium dieses Schadens, die LD50(30), setzt den Rahmen fest, innerhalb dessen «Erholung» hier gemessen werden kann. Die LD50(30) ist bekanntlich diejenige Strahlendosis, die 50% einer Population innerhalb von 30 Tagen tötet. Nur diejenigen Strahlenschäden, die zum tödlichen Ausgang innerhalb von 30 Tagen beitragen, werden also erfasst. Wenn die LD50(30) ein Mass für alle Strahlenschäden darstellt, deren Summe innerhalb von 30 Tagen tödlich wirkt, so kann die Split-Dosis-Methode als Mass für die Erholung von eben diesen Strahlenschäden gelten. Das ermöglicht eine formale Definition der Erholung als Abnahme eines von einer Erstdosis herrührenden Restschadens, der durch die Differenz der LD50(30)-Werte von vorbestrahlten und nicht vorbestrahlten Tieren messbar wird[1].

Zunächst suchte man experimentelle Bestätigung für die theoretisch naheliegende Hypothese eines mit der Zeit exponentiell abnehmenden Strahlenschadens. Das Ergebnis hing weitgehend von der Wahl der Testzeiten nach Erstbestrahlung ab. Die Beobachtungen waren mit der Hypothese vereinbar, wenn sie etwa 2 Tage bis 3 Wochen nach der Erstbestrahlung gesammelt wurden[2–4]. Sie wichen aber in den ersten 48 h deutlich vom exponentiellen Verlauf ab [3,5–8]. Es kam dann zu einer Fluktuation des von der Erstbestrahlung zurückgebliebenen Restschadens, wobei regelmässig zuerst ein Minimum und dann ein Maximum auftrat. Das fand allgemeine Beachtung, nachdem Elkind und Sutton[9] an bestrahlten Hamsterzellkulturen in vitro eine ähnliche Fluktuation gefunden und durch das «Repair-and-progression»-Modell[10] gedeutet hatten. Seither wurden solche Verläufe bei bestrahlten zellulären Systemen vielfach nachgewiesen[11].

Im vorliegenden Split-Dosis-Experiment ging es um die formale Beschreibung der Erholungsvorgänge bei jungen Wistar-Ratten. Es war zu prüfen, ob die bisher nur an Mäusen beschriebene frühe Wellenbewegung sich auch an Ratten nachweisen liess.

Methodik. Als Versuchstiere dienten Wistar-Ratten eigener Zucht, die in transparenten Plastik-Käfigen gehalten und mit Altromin Pellets und Wasser ernährt wurden. Zusätzlich gab es bis zum 30. Lebenstag rohes Fleisch und gelegentlich Möhren oder Salat. Die Raumtemperatur lag bei 22–26 °C, die relative Luftfeuchtigkeit bei 50–65%.

Die Bestrahlung erfolgte im Alter von 12, 24 oder 32 Tagen. Es kamen nur Würfe zum Versuch, die am Tag 12 mindestens 7 gesunde Jungtiere enthielten. Diese wurden am Tag 12 oder 24 zusammen bestrahlt und danach der Mutter zurückgegeben. In der ältesten Gruppe wurden die Muttertiere 4 Tage vor der Bestrahlung entfernt und die Jungen gleichzeitig nach dem Geschlecht getrennt und randomisiert.

Die Bestrahlungsbedingungen sind anderswo ausführlich beschrieben[12]. Die Tiere erhielten 1 oder 2 Röntgenganzbestrahlungen (200 kV, 20 mA, HWS 0,81 mm Cu, 108–116 R/min), wobei die Dosis zur Hälfte im dorsoventralen, zur Hälfte im ventrodorsalen Strahlengang appliziert wurde. Die Dosierungsinhomogenität des Bestrahlungsfeldes lag während der Versuchsdauer (April bis Oktober 1965) bei $\pm$ 1,75% Standardabweichung von einer Testdosis 65 R (der Messfehler betrug $\pm$ 1,86%).

In jedem Alter wurde ein unbestrahltes, ein einmal bestrahltes und ein zweimal bestrahltes Kollektiv benötigt. Das erste diente zur Schätzung der «natürlichen» Sterblichkeit, das zweite zur Schätzung der LD50(30) ohne Vorbestrahlungsdosis, das dritte zur Schätzung der

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