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The Complete Structure of Spathulin, a Crystallographic Study of Diacetylspathulin[1]

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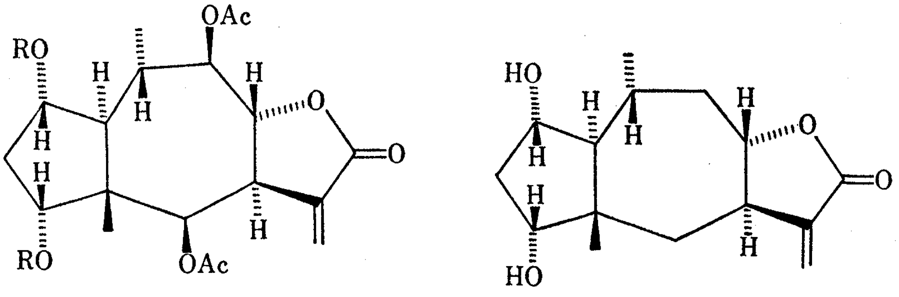
The molecular structure of spathulin (**1a**), a highly oxygenated pseudoguaianolide isolated from *Gaillardia grandiflora* has been established by a three dimensional X-ray crystallographic analysis of diacetylspathulin (**1b**). The space group of the crystal of **1b** is $P2_12_12_1$ and the dimensions of the cell with $z=4$ are $a=9.6976(3)$, $b=26.968(2)$, $c=8.9288(3)$ Å. The structure of the crystal was refined to $R=0.05$. The conformation of spathulin (**1a**) was estimated to be similar to that of pulchellin (**2a**), namely bis-deacetoxyspathulin except some differences particularly at C(6) and C(9), when the conformation of the pseudoguaianolide skeleton in **1b** is compared with that in 11,13-dibromopulchellin (**2b**).

Keywords——*Gaillardia grandiflora*; pseudoguaianolide; complete structure; spathulin; absolute configuration; diacetylspathulin; X-ray analysis; direct method; conformational comparison; 11,13-dibromopulchellin

A highly oxygenated pseudoguaianolide, spathulin (**1a**) was isolated from Gaillardia species of the Compositae plant, especially *G. spathulata*.[3] A nuclear magnetic resonance (NMR) spectrometric study of spathulin has deduced its relative stereochemistry as depicted in **1a**.[4] The absolute configulation and the ring conformation of the closely related compound, pulchellin (**2a**),[5] which was isolated from the related species *G. pulchella*, has already been established by X-ray crystallographic studies.[6]



1a: R=H; **1b**: R=Ac **2a**;**2b**: 11β-Br,13-Br

Formulae of Spathulin (**1a**), Diacethlspathulin (**1b**), Pulchellin (**2a**) and 11,13-Dibromopulchellin (**2b**)

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Spathulin (**1a**) is conceived to possess absolute β-configuration concerning the angular methyl group of pseudoguaianoids like in pulchellin (**2a**) on the basis of biogenetic considerations. However, the structural correlation between **1a** and **2a** has not yet been made due to a matter of considerable difficulty by chemical means. In view of our pharmacochemical interests in these sesquiterpenolides, the situation mentioned above prompted us to establish the complete structure of spathulin (**1a**), particularly the absolute configuration by an X-ray crystallographic analysis of **1a** or of an appropriate derivative. The material of **1a** isolated from a Japanese cultivar of *G. grandiflora* was in accordance with an authentic sample of spathulin7) by mixed melting points and comparison of optical rotations, circular dichroism (CD) curves, infrared (IR) and NMR spectra. Attempts to prepare suitable crystals of **1a** and its several derivatives for the X-ray crystallographic study failed in our hands except that of diacetylspathulin (**1b**).3) Colorless prisms of **1b** for the X-ray study was prepared by recrystallizations from the mixed solution of acetone and petroleum ether.

The present paper describes the molecular structure of **1b** established by the X-ray analysis on the single crystal of **1b**, and a discussion on the conformation of **1b** in the crystalline state thus revealed, which is quite similar to that of 11,13-dibromopulchellin (**2b**) with some differences between them.

Experimental

The melting points were taken on a Büchi (capillary tube) apparatus and are uncorrected. The IR spectra were recorded on a Hitachi EPI-G3 instrument, ^{1}H NMR spectra were measured on a JEOL MH-100 spectrometer(100 MHz) and the ultraviolet(UV) spectra were determined on a Shimadzu UV-200 spectrophotometer. Optical rotations were run on a JASCO DIP-180 automatic polarimeter. The high resolution mass spectra (MS) were measured on a JEOL JMS-01SG at 75 eV (ion source temperature was 260°).

Isolation of Spathulin (1a)——Above-ground, dried, chipped whole plant of a cultivar of *G. grandiflora* harvested in 1972 (13.5 kg) were extracted with hot trichloroethane, and the extract was freed from chlorophil by the ordinary method. It is of interest to note in the present case that the crude crystals (1.02 g) (0.0075% based on the dried material) separated from the aqueous layer on treatment of the extract with a hot Pb-$(OAc)_2$ solution. Repeated recrystallizations from several solvent combinations and finally from acetone–petroleum ether yielded colorless needles of pure spathulin (**1a**). $C_{19}H_{26}O_8$ (MS *m/e*: 382.402 (M^+). *Anal.* Found: C, 59.59; H, 7.14. Calcd.: C, 59.67; H, 6.85). mp 256—258°. $[\alpha]_D^{28}$ +10° (EtOH; *c*, 1.0). UV λ_{max}^{EtOH}: 209 nm (ε 12066). IR ν_{max}^{KBr} cm^{-1}: 3495 and 3318 [O(2)–H and O(4)–H], 1781 [C(12)=O(10)], 1743 and 1726 [C(20)=O(7) and C(22)=O(9)], 1256 and 1241 [C(6)–O(6)–C(20), C(8)–O(1)–C(12) and C(9)–O(8)–C(22)], 1668 [$C(11)=C(13)H_2$]. NMR (DMSO-d_6) δ ppm (TMS): 0.64 (s, 3, C-15 methyl), 1.07 (d, 3, $J=6$ Hz, C-14 methyl), 1.96 and 2.08 (s, 6, C-21 methyl and C-23 methyl), 3.51 (m, 1, C-7 methine), 3.55 (d, 1, $J=4$ Hz, C-4 methine), 3.95 (m, 1, C-2 methine), 4.46 (m(t), 2, C-8 methine and O-2 hydrogen), 4.86 (m(t), 2, C-9 methine and O-4 hydrogen), 5.43 (d, 1, $J=3$ Hz, C-13 methylene), 5.98 (d, 1, $J=2$ Hz, C-6 methine), 6.09 (d, 1, $J=3$ Hz, C-13 methylene). CD (EtOH; *c*, 0.43) $(\theta)_{300}$ +21, $(\theta)_{280}$ +150, $(\theta)_{270}$ +207 (max), $(\theta)_{260}$ +111, $(\theta)_{255}$ +15, $(\theta)_{254}$ 0, $(\theta)_{245}$ −210, $(\theta)_{230}$ −615, $(\theta)_{220}$ −1155. Our sample mentioned above was within experimental errors identical with an authentic sample7) on comparison of respective spectral data of spathulin.

Diacetylspathulin (1b)——Spathulin (**1a**) was acetylated with Ac_2O and pyridine by the usual manner. Several recrystallizations from acetone–petroleum ether gave colorless prisms of diacetylspathulin (**1b**). $C_{23}H_{30}O_{10}$ (MS *m/e*: 466.473 (M^+), *Anal.* Found: C, 59.23; H, 6.69. Calcd.: C, 59.22; H, 6.48). mp 252.5—254.5°. $[\alpha]_D^{28}$ −15.4° ($CHCl_3$; *c*, 0.513). UV λ_{max}^{EtOH}: 208 nm (ε 9868). IR ν_{max}^{KBr} cm^{-1}: 1777 [C(12)=O(10)], 1742, vs, [C(16)=O(3), C(18)=O(5), C(20)=O(7) and C(22)=C(9)], 1254 and 1242 [C(2)–O(2)–C(16), C(4)–O(4)–C(18), C(6)–O(6)–C(20), C(8)–O(1)–C(12) and C(9)–O(8)–C(22)], 1669 [C(11)=C(13)]. NMR ($CDCl_3$) δ ppm (TMS): 0.89 (s, 3, C-15 methyl), 0.98 (d, 3, $J=7$ Hz, C-14 methyl), 1.98, 2.00, 2.11 and 2.13 (s, 12, C-17 methyl, C-19 methyl, C-21 methyl and C-23 methyl), 3.24 (m, 1, C-7 methine), 4.99 (m, 4, C-2 methine, C-4 methine, C-8 methine and C-9 methine), 5.48 (d, 1, $J=3$ Hz, C-13 methylene), 5.79 (d, 1, $J=3$ Hz, C-6 methine), 6.30 (d, 1, $J=3$ Hz, C-13 methylene). The physical properties of **1b** described above were almost in accordance with those reported previously3) except undetermined MS and UV data.

Collection of the Crystal Data and Intensity Data——Crystals of diacetylspathulin (**1b**) for the X-ray study were grown from acetone solutions as colorless transparent prisms elongated along the *b* axis. The single crystal with approximate dimensions of 0.35×0.32×0.42 mm was chosen for the X-ray diffraction

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7) A sample of spathulin and certain spectral data were supplied by a courtesy of Prof. W. Herz of Florida State University to whom we are indebted.

Table I. Crystal Data

Diacetylspathulin: $C_{23}H_{30}O_{10}$, MW=466.47, mp=252.5—254.5°
Orthorhombic, Space group $P2_12_12_1$, $Z=4$
$a=9.6976$ (3), $b=26.968$ (2), $c=8.9288$ (3) Å

study. Intensities and the cell parameters were measured on a Philips four-circle X-ray diffractometer with graphite monochromated CuKα radiation. The crystal data are listed in Table I. A total of 3979 reflexions (including Friedel reflexions mentioned below) were measured within $2\theta=156°$ using the θ-2θ scanning technique, in which 320 were too weak to be observed. The scanning speed was 4° min^{-1} in θ and the scans were repeated twice when the total number of counts received in a single scan was less than 10^3. 845 Friedel reflexions ($\bar{h}\bar{k}\bar{l}$ *vs.* hkl) were measured immediately after the measurement of hkl. These intensity data were then corrected for Lorentz and polarization factors and converted to the normalized structure factors, E. The number of independent structure factors was 2814 excluding the Friedel reflexions.

Crystal Structure Analysis——The structure was solved by the direct method using the program MULTAN by Main, Woolfson and Germain.[8] Starting with 9 reflexions the 369 phases having E values greater than 1.5 were determined and refined by using the tangent formula. One of the trial sets giving the absolute figure of merit of 1.03 and the R factor of 37.4% revealed the correct disposition of the atoms. This structure was then refined by the least-squares method coupled with Fourier and difference Fourier syntheses. All the heavier atoms along with 18 hydrogen atoms out of 30 were located. Remaining 12 hydrogen atoms were those involved in the four terminal acetyl groups. The final conventional R factor was 0.05.

The determination of the absolute configuration was attempted by taking into account the anomalous dispersion effect of the oxygen atoms for CuKα radiation. The dispersion corrections, $\Delta f'$ and $\Delta f''$ were assumed to be 0.047 and 0.032 (International Tables for X-ray Crystallography, Vol. IV, 1975). Pairs of Friedel reflections were selected for which the difference between the observed $|F(hkl)|$ and $|F(\bar{h}\bar{k}\bar{l})|$ was greater than $3\{\sigma^2(Fo(hkl)+\sigma^2(Fo(\bar{h}\bar{k}\bar{l}))\}^{1/2}$ and the corresponding calculated values differed more than 2%. Comparison of the calculated and observed values of $|F(hkl)|$ and $|F(\bar{h}\bar{k}\bar{l})|$ for these pairs (Table II) suggested that the coordinate system adopted in the measurement of the intensities must be inverted to describe the correct absolute configuration. This configuration is in accordance with that determined for 11,13-dibromopulchellin by X-ray anomalous dispersion method. All the Figures and Diagrams given in the present paper are drawn with the suggested coordinate system.

Table II. Comparison of the Calculated and Observed Values of $|F(hkl)|$ and $|F(\bar{h}\bar{k}\bar{l})|$

h	k	l	$\lvert Fc\ (hkl)\rvert$		$\lvert Fc\ (\bar{h}\bar{k}\bar{l})\rvert$	$\lvert Fo\ (hkl)\rvert$		$\lvert Fo\ (\bar{h}\bar{k}\bar{l})\rvert$	$\sigma\ (Fo)$
3	18	1	4.04	<	4.16	5.72	>	4.66	0.3
2	2	6	4.78	<	4.85	4.77	>	3.45	0.3
5	6	6	3.69	<	3.86	4.50	>	3.42	0.3
6	12	6	2.76	<	2.79	2.94	>	1.46	0.4
3	13	6	7.24	<	7.39	7.72	>	6.69	0.3
6	17	6	2.50	<	2.56	2.85	>	1.59	0.4
2	2	7	3.69	<	3.85	4.81	>	3.92	0.3
7	1	8	5.31	<	5.36	6.08	>	5.39	0.3
7	2	8	6.81	>	6.73	6.42	<	7.14	0.3
6	5	9	3.80	<	3.87	3.63	>	2.92	0.3

Only those reflexions are listed for which the observed $|F\ (hkl)|$ and $|F\ (\bar{h}\bar{k}\bar{l})|$ differ more than 3σ (Fo) and the calculated $|F\ (hkl)|$ and $|F\ (\bar{h}\bar{k}\bar{l})|$ differ more than 2%.

Results and Discussion

The final atomic parameters are listed in Table III. Bond lengths (in angstrom unit) and angles (in degrees) are shown in Fig. 1 and Fig. 2. The average standard deviations of these values are estimated to be: 0.006 Å for C–C, and 0.05 Å for C–H lengths and 0.3° for C–C–C and about 3° for C–C–H angles.

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TABLE III. Final Atomic Parameters

	x	y	z	$\beta 11$	$\beta 22$	$\beta 33$	$\beta 12$	$\beta 13$	$\beta 23$
C (1)	6269(4)	1168(1)	2892(4)	69 (4)	7(0)	87 (4)	−1(1)	2 (4)	0(1)
C (2)	6831(4)	1640(1)	3669(4)	74 (4)	9(0)	97 (5)	−1(1)	−3 (4)	−4(1)
C (3)	6244(4)	2091(1)	2855(5)	105 (5)	9(1)	116 (5)	2(1)	−8 (5)	−4(1)
C (4)	5689(4)	1885(1)	1410(5)	90 (4)	8(0)	107 (5)	1(1)	3 (4)	2(1)
C (5)	5109(4)	1370(1)	1839(4)	71 (4)	8(0)	90 (4)	1(1)	1 (4)	1(1)
C (6)	4912(4)	1060(1)	409(4)	78 (4)	8(0)	88 (4)	−4(1)	5 (4)	3(1)
C (7)	5032(4)	501(1)	609(4)	69 (4)	10(0)	87 (4)	−1(1)	1 (4)	−1(1)
C (8)	4464(4)	272(1)	2054(4)	83 (4)	7(0)	96 (5)	−2(1)	4 (4)	−1(1)
C (9)	5565(4)	250(1)	3267(4)	88 (4)	9(0)	83 (4)	−1(1)	2 (4)	6(1)
C (10)	5899(4)	753(1)	4020(4)	87 (4)	9(0)	89 (5)	−1(1)	−8 (4)	2(1)
C (11)	4402(4)	185(1)	−594(5)	98 (4)	10(1)	103 (5)	1(1)	−7 (4)	−4(1)
C (12)	3905(5)	−276(2)	137(5)	111 (5)	12(1)	132 (6)	−2(2)	−13 (5)	−4(2)
C (13)	4259(6)	261(2)	−2038(5)	173 (7)	15(1)	111 (6)	3(2)	−11 (6)	−7(2)
C (14)	7087(5)	667(2)	5117(5)	132 (6)	13(1)	131 (6)	−7(2)	−43 (5)	10(2)
C (15)	3736(4)	1463(1)	2673(5)	77 (4)	12(1)	116 (5)	4(1)	17 (4)	0(2)
C (16)	9067(4)	1975(1)	4216(5)	100 (5)	13(1)	95 (5)	−9(1)	−6 (4)	−2(2)
C (17)	10583(5)	1916(2)	3865(6)	82 (5)	20(1)	183 (8)	−11(2)	6 (5)	−12(2)
C (18)	7241(5)	2163(1)	−496(5)	156 (6)	11(1)	109 (6)	−12(2)	−5 (6)	6(2)
C (19)	8369(7)	1995(2)	−1521(6)	235(10)	17(1)	163 (8)	−17(2)	93 (8)	2(2)
C (20)	3596(7)	1430(2)	−1576(6)	231 (9)	14(1)	158 (8)	−6(2)	−91 (8)	15(2)
C (21)	2124(7)	1491(2)	−2149(7)	248(11)	18(1)	236(11)	14(3)	−162(10)	0(3)
C (22)	5716(5)	−514(1)	4651(5)	118 (5)	10(1)	129 (6)	2(1)	−13 (5)	7(2)
C (23)	4987(7)	−831(2)	5776(6)	219 (9)	10(1)	152 (7)	2(2)	24 (8)	15(2)
O (1)	4091(3)	−238(1)	1646(3)	111 (3)	9(0)	126 (4)	−8(1)	−11 (3)	1(1)
O (2)	8322(3)	1617(1)	3522(3)	71 (3)	11(0)	126 (4)	−4(1)	−7 (3)	−8(1)
O (3)	8550(3)	2288(1)	4975(3)	129 (4)	17(0)	131 (4)	−8(1)	4 (4)	−19(1)
O (4)	6842(3)	1795(1)	404(3)	98 (3)	9(0)	111 (3)	−5(1)	12 (3)	5(1)
O (5)	6747(5)	2574(1)	−438(4)	271 (7)	11(0)	184 (6)	−4(1)	39 (6)	15(1)
O (6)	3583(3)	1176(1)	−247(3)	108 (3)	10(0)	115 (4)	3(1)	−40 (3)	4(1)
O (7)	4634(6)	1563(2)	−2174(5)	295 (9)	41(1)	220 (7)	−47(3)	−86 (7)	61(3)
O (8)	5035(3)	−81(1)	4434(3)	112 (3)	8(0)	102 (3)	1(1)	9 (3)	9(1)
O (9)	6772(4)	−619(1)	4017(5)	140 (4)	16(1)	268 (7)	15(1)	32 (5)	22(2)
O (10)	3436(4)	−643(1)	−424(4)	221 (6)	12(0)	183 (5)	−18(1)	−44 (5)	−11(1)
H (C1)	705(5)	104(1)	222(5)	4 (1)					
H (C2)	655(5)	164(1)	477(5)	4 (1)					
H (C3)	700(4)	235(1)	266(5)	4 (1)					
H′ (C3)	555(5)	224(2)	345(6)	6 (1)					
H (C4)	511(4)	208(1)	90(4)	3 (1)					
H (C6)	558(4)	115(1)	−34(5)	4 (1)					
H (C7)	590(5)	48(2)	59(5)	4 (1)					
H (C8)	356(4)	47(1)	244(4)	3 (1)					
H (C9)	651(4)	12(1)	281(5)	3 (1)					
H (C10)	497(4)	87(1)	470(5)	4 (1)					
H (C13)	372(6)	2(2)	−266(7)	7 (1)					
H′ (C13)	466(6)	58(2)	−246(6)	7 (1)					
H (C14)	681(5)	40(2)	568(6)	6 (1)					
H′ (C14)	722(5)	98(2)	567(6)	6 (1)					
H″ (C14)	788(5)	62(2)	454(6)	6 (1)					
H (C15)	302(5)	112(2)	276(6)	7 (1)					
H′ (C15)	311(5)	171(2)	215(6)	5 (1)					
H″ (C15)	395(5)	160(2)	375(6)	6 (1)					

For non-hydrogen atoms: x, y, z and β's are multiplied by 10^4. Temperature factors are of the from, $T=\exp\{-(\beta_{11}h^2+\beta_{22}k^2+\beta_{33}l^2+2\beta_{12}hk+2\beta_{13}hl+2\beta_{23}kl)\}$. For hydrogen atoms: x, y and z are multiplied by 10^3.

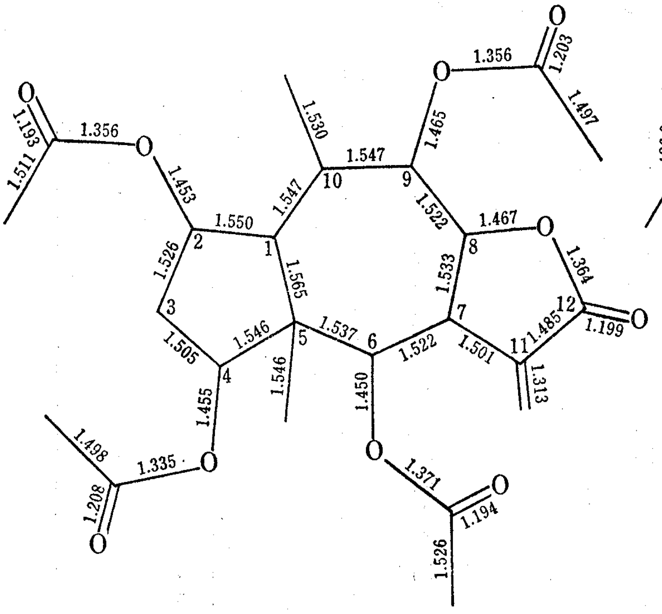


Fig. 1. The Bond Lengths (Å) in the Molecule of Diacetylspathulin (**1b**)

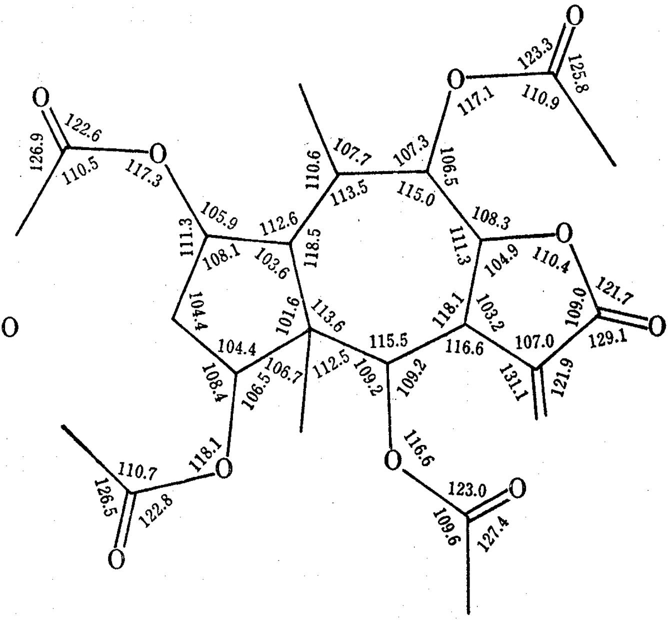


Fig. 2. The Bond Angles (Å) in the Molecule of Diacetylspathulin (**1b**)

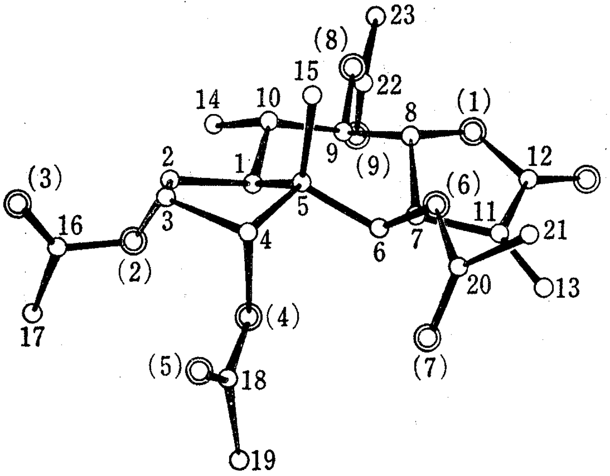


Fig. 3(a). Perspective View of the Molecule of Diacetylspathulin (**1b**)

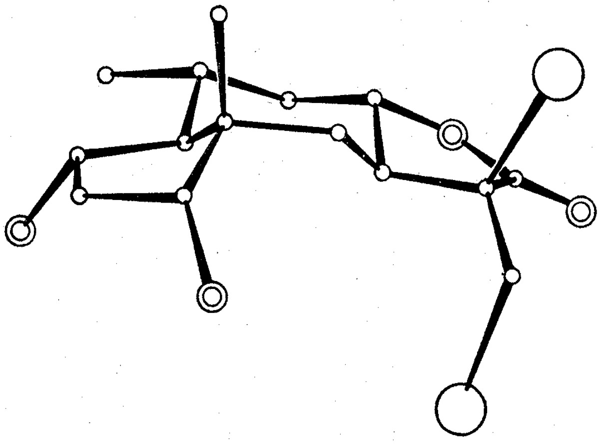
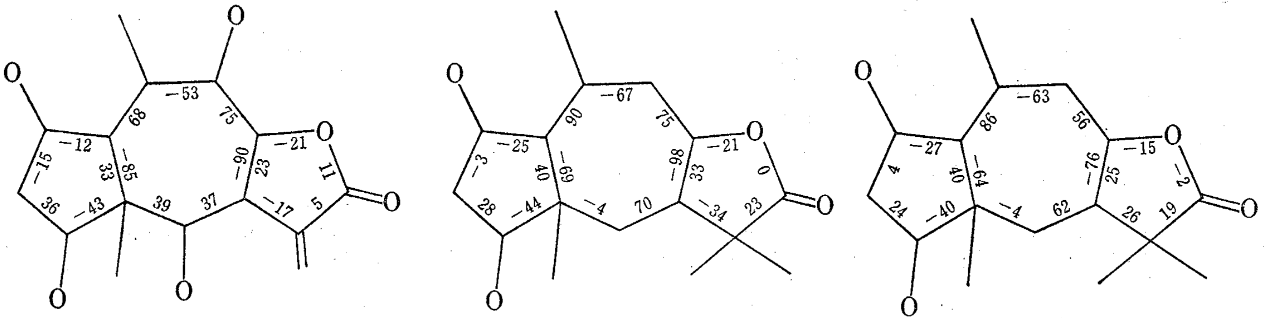


Fig. 3(b). Perspective View of the Molecule of 11,13-Dibromopulchellin (**2b**)



(a) (b) (c)

Fig. 4. Endocyclic Torsion Angles (Å). (a) Diacetylspathulin (**1b**), (b) 11,13-Dibromopulchellin (**2b**), Molecule I, (c) 11, 13-Dibromopulchellin (**2b**), Molecule II

The configuration and conformation of the molecule of diacetylspathulin (**1b**) are shown in Fig. 3(a). The seven-membered ring of the molecule takes a distorted flat chair conformation in order to release the nonbonded interference between C(5)-β methyl and C(10)-β hydrogen like in pulchellin (Fig. 3(b)).[6a,b] The conformations of pseudoguaianolide skeleton in the present compound, namely 6β,9β-diacetoxypulchellin (**1b**) and in 11,13-dibromopulchellin (**2b**)[6a,b] are compared in Fig. 4 showing the endocyclic torsion angles (in degrees) along the bonds forming the ring. The difference in the two conformations can be seen on comparison of Fig. 3 with Fig. 5(a) shown in the preceding paper,[6b] in both of which the conformations are drawn by putting the molecules nearly in the same orientation. The respective bond lengths and angles in the two compounds are very similar and the differences, do not exceed those found between the two crystallographically independent molecules, I and II,[6b] in 11,13-dibromopulchellin (**2b**), except for the bonds involving the atoms C(6), C(9) and C(11) which are chemically nonequivalent in these compounds. Some differences however, are found in the conformation of the skeletons. As seen in the endocyclic torsion angles about C(5)–C(6), the state of puckering in the seven-membered ring is different at the C(6) atom. This is reasonably attributable to the steric repulsions among the bulky quasi-axial groups at C(5), C(6) and C(9) in diacetylpulchellin (**1b**). Another difference in the conformations is observed at C(11) of each γ-lactone ring, which reflects the difference in the valence state of the C(11) atoms in these compounds.

The full structure including the absolute configuration of spathulin (**1a**) has now been established as designated above. The favored conformation of **1a** is probably similar to the present compound (**1b**), and the complete structure deduced by the present work confirms the relative stereochemistry assigned by Herz, *et al.*[4]

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