

THE STRUCTURES OF TWO ISOMERIC N-FORMATES FROM
3-O-ACETYLSOLASODINE

Genjiro Kusano, *^a Chika Sekimoto,^a Mayumi Abe,^a
Yasuko In,^a Hirofumi Ohishi,^a Toshimasa Ishida,^a
and Norio Aimi^b

- a) Osaka University of Pharmaceutical Sciences,
2-10-65 Kawai, Matsubara, Osaka 580, Japan
b) Faculty of Pharmaceutical Sciences, Chiba
University, 1-33 Yayoi-cho, Chiba 260, Japan

Abstract----- The structures of two isomeric N-formates, I (1) and II (2), prepared from O-acetylsolasodine, were determined by X-ray crystallographic analyses, and the isomerism between them was shown to be caused by the epimerization at C-22 during the formylation.

Solasodine, a common Solanum alkaloid, has been known to provide two N-formyl derivatives, whose isomerism was proposed to be due to restricted rotation of the C \equiv N partial double bond of the amide,¹ nitrogen inversion,² and an epimerization at C-22.³ In this report, X-ray crystallographic analyses of O-acetyl-N-formylsolasodine isomers I (1) and II (2) led us to the conclusion that the isomerism is due to an epimerization at C-22. Solasodine, (22R,25R)-22-N-spirosol-5-en-3 β -ol,⁴ is known to be teratogenic in contrast to tomatidine, (22S,25S)-22-N-spirosolan-3 β -ol.⁵ Since a mixture of the isomeric N-formylsolasodines showed teratogenic activity,^{6,7} a definitive structural determination was sought for both the N-formates to establish the relationships of molecular structures of the Solanum

alkaloids and their teratogenic activity.⁸

RESULTS AND DISCUSSION

Upon treatment with acetic-formic anhydride in chloroform at room temperature, *O*-acetylsolasodine yielded two isomeric *N*-formates, I (1), mp 203 - 205°, C₃₀H₄₅NO₄, and II (2), mp 194 - 196°, C₃₀H₄₅NO₄. I and II were identical to the previously reported *N*-formates after comparison of thin layer chromatography (tlc) and ¹H-nuclear magnetic resonance (nmr) spectra.² ¹H-Nmr signals due to *N*-formyl groups and *N*-methylene groups of I and II were identical with those of the major isomer (3) and the minor (4) of diformylsolasodines, respectively, reported by W. Gaffield *et al.*³ Therefore, the structures of I and II should be same with those of the former and the latter except the 3β-*O*-acyl groups.

The crystals of I and II were grown in an ethyl acetate solution as colorless plates and were found suitable for X-ray analysis. The crystal data are summarized in Table I. The structures were solved by a direct method with the aid of the program SHELXS-86 and then refined by the least-squares method. The final R-factors were 4.99% for 2504 observed reflections for I and 6.37% for 2455 for II. The structures of I and II are illustrated in Figure 1 by an ORTEP drawing. Thus, the structures of I and II were concluded to be (22R,25R)-*N*-formyl-22-*N*-spirosol-5-en-3β-yl acetate and (22S, 25R)-*N*-formyl-22-*N*-spirosol-5-en-3β-yl acetate. The torsional angles concerning the stereochemistry at C-22 and the rings E and F (Table II) showed 22R for I and 22S for II, and deformed rings for E and chair forms for F.

This conclusion is same to that proposed by W. Gaffield *et al.*³ Analyses of mass spectra, ¹³C and ¹H-nmr spectra of 3 and 4 led to this conclusion, also proposing that the preferred ring F conformations of 3 and 4 possess twist-boat by the conformational analysis and other considerations as shown in Figure 3. It is notable that this X-ray analysis showed that the ring Fs of I and II adopt chair-like conformation as shown in Figure 2.

It is also notable that the ring E of I possesses half-chair, while that of II twist conformation. The level (\AA) of each atom of the ring E from the best plane calculated according to the plane equations is shown in Figure 4. The equations were obtained from the results of the X-ray crystallographic analysis as follows :

$$0.19178 X + 0.72677 Y - 0.65957 Z - 3.09891 = 0 \text{ for I,}$$

$$- 0.16240 X + 0.89402 Y - 0.41757 Z + 3.50285 = 0 \text{ for II.}$$

The computer drawing of the stereostructures of I and II shows that the rings A, B, C and D are superimposable, while the rings E and F not. These observations suggest that strain due to interaction between C-21 methyl and N-formyl group for I and C-21 methyl and C-23 methylene group for II may be relieved by adopting a half-chair form for the ring E in I and a twist form in II.

According to the hypothesis that steroidal alkaloids bearing a basic nitrogen atom in the ring F, with bonding capabilities α to the steroid plane are teratogens, the isomer I may be a principle of the teratogenicity recognized with a mixture of isomeric N-formylsolasodines.

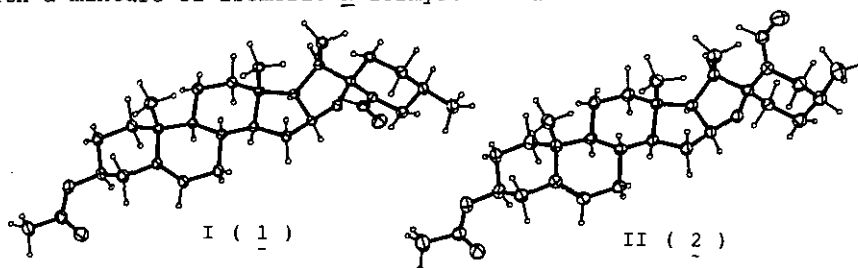


Figure 1. ORTEP Drawings of I (1) and II (2)

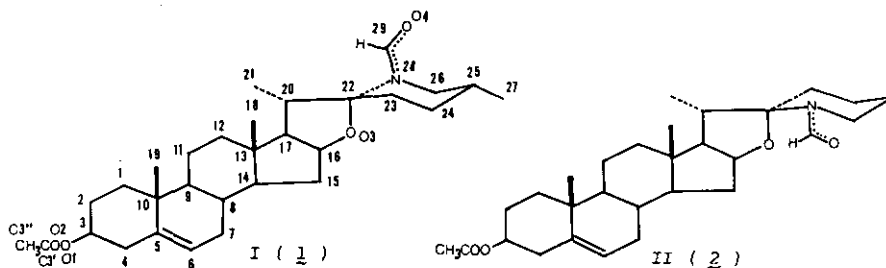


Figure 2. Molecular Structures of I and II

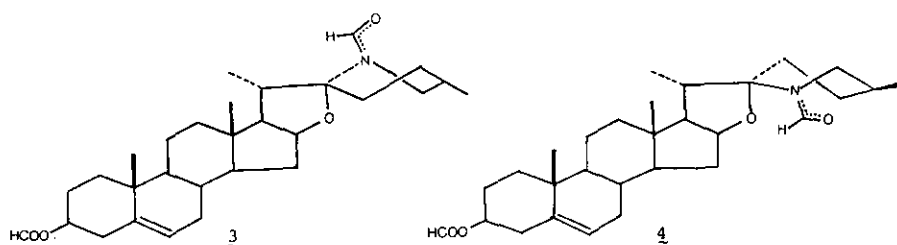


Figure 3. Structures of 3 and 4 Proposed by W. Gaffield et al.

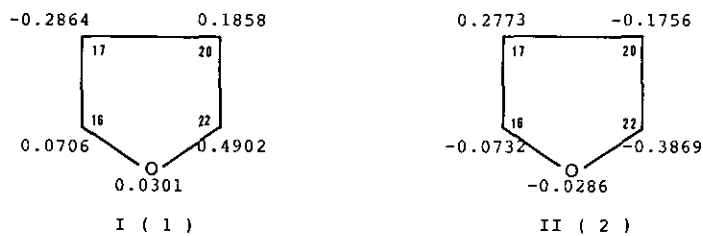


Figure 4. The level (Å) of each atom of ring E from the best plane.

Table I. Crystal Data of I (1) and II (2)

Compound	I (<u>1</u>)	II (<u>2</u>)
Molecular Formula	C ₃₀ H ₄₅ NO ₄	C ₃₀ H ₄₅ NO ₄
Crystal System	monoclinic	orthorhombic
Cell Constants		
a (Å)	17.9430(2)	35.1700(7)
b	6.6560(1)	10.2680(9)
c	11.4750(1)	7.4900(3)
V (Å ³)	1357.4(3)	2704.0(26)
Space Group	P2 ₁	P2 ₁ 2 ₁ 2 ₁
Z Value	2	4
Dx g/cm ⁻³	1.1835	1.1878
X-ray λ (CuKα)	1.5418	1.5418
F (0 0 0)	528	1056

EXPERIMENTAL

Melting points were measured on Yanagimoto micromelting point apparatus and uncorrected. The nmr spectra were determined with Varian Gemini-200 and Varian XL-300 spectrometers with tetramethylsilane as the internal standard and the chemical shift are expressed in δ values. Mass spectra were taken on a JEOL JMS-DX 300 mass spectrometer.

The preparation of O-acetylsolasodine N-formates I (1) and II (2)

O-Acetylsolasodine (1.0 g, 2 m mol)¹⁰ was dissolved in CHCl_3 (50 ml) and newly prepared acetic-formic anhydride (6 ml, 40 m mol, not titrated) was added to the solution dropwise under cooling in an ice-bath. After stirring for 30 min at room temperature, water (ca. 20 ml) and then sodium carbonate (powder, until no bubbling CO_2) were added to the reaction solution under cooling in ice-bath. The CHCl_3 solution was washed with water three times and dried over Na_2SO_4 overnight. After removal of the drying reagent and evaporation of the solvent under vacuo, the residue was subjected to flash chromatography on Kieselgel H (50 g, ϕ 33 x 180 mm) with hexane-AcOEt (8 : 1) to provide I (1) 155 mg, II (2) 127 mg and their mixture 675 mg. The first product I, mp 203 - 205°. Ms: m/z 495 (M^+), $\text{C}_{30}\text{H}_{45}\text{NO}_4$. Nmr(CDCl_3)ppm: δ 8.42 (1H,s,N-CHO), 5.34(1H,d,J=6 Hz,H-6), 4.56 (1H,m,H-3), 4.18 (1H,dd, $J_1=12$ Hz, $J_2=2$ Hz, H-26eq.), 4.15 (1H,dd, $J_1=15$ Hz, $J_2=7$ Hz, H-16), 2.27 (1H,dd, $J_1=J_2=12$ Hz, H-26ax.), 1.99 (3H, s, COCH₃), 1.13 (3H,d,J=7 Hz, H-27), 1.00 (3H,s, H-19), 0.89 (3H, d, $J=7$ Hz, H-21), 0.80 (3H,s, H-18). Nmr(pyr-d₅)ppm: δ 4.40 (1H,dd, $J_1=13$ Hz, $J_2=3$ Hz, H-26eq.), 4.16 (1H,dd, $J_1=8.5$ Hz, $J_2=15$ Hz, H-16), 2.39 (1H,dd, $J_1=J_2=13$ Hz, H-26ax.). The second product II, mp 194 - 196°. Ms: m/z 495 (M^+), $\text{C}_{30}\text{H}_{45}\text{NO}_4$. Nmr(CDCl_3)ppm: δ 8.62 (1H,s, N-CHO), 5.35(1H, d, $J=6$ Hz, H-6), 4.58 (1H, m, H-3), 4.27 (1H, dd, $J_1=6$ Hz, $J_2=15$ Hz, H-16), 3.85 (1H, dd, $J_1=14$ Hz, $J_2=3$ Hz, H-26eq.), 3.11 (1H, dd, $J_1=14$ Hz, $J_2=7$ Hz, H-26ax.).

Collection of the Crystal Data and Intensity Data

The crystals of I and II were grown in an AcOEt solution as colorless

plates. The lattice constants and intensity data were measured on a Rigaku AFC-5 diffractometer with graphite monochromated Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$). The crystal data are summarized in Table I. A total of 2504 independent reflections for I and 2455 for II were measured in an $\omega - 2\theta$ scan mode to $2\theta \text{ max} = 130^\circ$ at 18°C . The intensities were corrected for the Lorentz and polarization effects.

Crystal Structure Analysis

The structures were solved by a direct method with the aid of the program SHELXS-86 and then refined by the least-squares method. The parameters were refined including the coordinates and anisotropic thermal parameters for all nonhydrogen atoms (Table III for I, Table IV for II). Ideal positions of hydrogen atoms, calculated on the basis of stereochemical considerations, were verified on a difference Fourier map and refined isotropically. The UNICS programs were used for crystallographic computation.

Semiempirical Energy Calculations

The total energy variations of I and II as a function of torsion angle θ were calculated by the CNDO/2 method. Table II shows torsional angles concerning the stereochemistry at C-22 and the conformation of the rings E-F.

Table II. Torsional Angles ($^\circ$) and Standard Deviations Concerning the Stereochemistry at C-22 and Ring E and F.

Bond	Angles		Bond	Angles	
	I	II		I	II
C14 - C15 - C16 - C17	20.6(3)	22.0(4)	C14 - C13 - C17 - C20	-147.8(3)	-148.9(4)
C14 - C15 - C16 - O3	136.1(3)	138.4(4)	O3 - C22 - C23 - C24	66.9(3)	-60.8(4)
C15 - C16 - C17 - C13	7.5(2)	7.7(3)	C20 - C22 - N28 - C26	-178.4(3)	174.0(4)
C15 - C16 - C17 - C20	134.2(3)	132.0(4)	C20 - C22 - N28 - C29	-17.1(3)	-3.4(5)
O3 - C16 - C17 - C13	-111.7(2)	-113.3(3)	C23 - C22 - N28 - C26	52.6(3)	-57.7(4)
O3 - C16 - C17 - C20	15.1(2)	11.0(3)	C23 - C22 - N28 - C29	-146.1(4)	124.9(6)
C15 - C16 - O3 - C22	-155.7(3)	-153.1(4)	O3 - C22 - N28 - C26	-64.4(3)	61.3(4)
C17 - C16 - O3 - C22	-38.5(2)	-34.9(4)	O3 - C22 - N28 - C29	96.9(4)	-116.1(5)
C13 - C17 - C20 - C21	-99.4(3)	-104.9(4)	C20 - C22 - O3 - C16	45.2(2)	44.3(4)
C13 - C17 - C20 - C22	127.2(3)	129.3(4)	C23 - C22 - O3 - C16	163.2(3)	-77.1(4)
C16 - C17 - C20 - C21	144.9(3)	140.2(4)	N28 - C22 - O3 - C16	-78.2(2)	165.4(3)
C16 - C17 - C20 - C22	11.4(2)	14.4(4)	C22 - C23 - C24 - C25	53.6(3)	-55.1(5)
C17 - C20 - C22 - C23	-148.3(3)	84.8(4)	C23 - C24 - C25 - C26	-52.8(3)	54.4(5)
C17 - C20 - C22 - N28	84.0(3)	-149.3(4)	C23 - C24 - C25 - C27	-175.1(4)	-73.1(5)
C17 - C20 - C22 - O3	-33.9(2)	-34.9(3)	C24 - C25 - C26 - N28	53.1(3)	-52.9(5)
C21 - C20 - C22 - C23	79.2(3)	-38.8(4)	C27 - C25 - C26 - N28	178.0(4)	73.2(5)
C21 - C20 - C22 - N28	-48.5(3)	87.1(4)	C25 - C26 - N28 - C22	-55.3(3)	57.8(5)
C21 - C20 - C22 - O3	-166.4(3)	-158.5(4)	C25 - C26 - N28 - C29	142.1(4)	-124.4(5)
C20 - C22 - C23 - C24	177.8(3)	-175.6(5)	C22 - N28 - C29 - O4	-172.6(6)	-179.5(8)
N28 - C22 - C23 - C24	-51.0(3)	55.0(4)	C26 - N28 - C29 - O4	-11.6(4)	3.2(6)

Table III. Positional ($\times 10^4$) and Thermal Parameters (\AA^2) of I for Non-hydrogen Atoms with Their Standard Deviations in Parentheses.

Atom	X	Y	Z	Req.
C1	6902(2)	8381(8)	4500(3)	4.5 (1)
C2	7777(2)	8529(8)	4617(3)	4.5 (2)
C3	8053(2)	6960(7)	3857(3)	4.4 (1)
C3'	9241(2)	5366(9)	3983(4)	5.5 (2)
C3''	10069(2)	5660(1)	4041(5)	7.0 (3)
C4	7749(2)	7346(8)	2563(3)	4.8 (2)
C5	6896(2)	7263(6)	2397(3)	3.7 (1)
C6	6534(2)	6051(7)	1614(3)	4.3 (1)
C7	5692(2)	5847(7)	1351(3)	4.4 (1)
C8	5278(2)	7503(6)	1914(2)	3.3 (1)
C9	5662(2)	7891(5)	3188(3)	3.3 (1)
C10	6494(2)	8606(6)	3211(3)	3.5 (1)
C11	5199(2)	9259(7)	3887(3)	4.2 (1)
C12	4356(2)	8641(7)	3809(3)	4.0 (1)
C13	3993(2)	8424(5)	2524(2)	3.2 (1)
C14	4466(2)	6910(5)	1946(2)	3.2 (1)
C15	3976(2)	6376(6)	787(3)	3.7 (1)
C16	3205(2)	6177(5)	1181(3)	3.3 (1)
C17	3216(2)	7318(5)	2351(2)	3.3 (1)
C18	3948(2)	10493(6)	1934(3)	3.9 (1)
C19	6532(3)	10818(7)	2812(4)	5.3 (2)
C20	2475(2)	8528(6)	2170(3)	3.3 (1)
C21	2062(2)	8823(7)	3249(3)	4.6 (2)
C22	2036(2)	7635(5)	1032(3)	3.3 (1)
C23	1542(2)	9185(6)	329(3)	4.1 (1)
C24	1125(2)	8346(7)	- 828(3)	4.7 (2)
C25	682(2)	6475(8)	- 636(3)	4.6 (2)
C26	1201(2)	4956(7)	69(3)	4.4 (1)
C27	314(2)	5500(1)	-1773(4)	5.7 (2)
N28	1599(2)	5815(5)	1167(2)	3.8 (1)
C29	1697(2)	4624(7)	2120(3)	4.5 (2)
O1	8862(1)	7065(5)	3981(3)	5.5 (1)
O2	3928(2)	3745(7)	3893(4)	6.1 (2)
O3	2635(1)	7147(6)	370(2)	3.58(8)
O4	1398(2)	3010(4)	2224(3)	6.7 (2)

REFERENCES

- 1) L. Toldy and L. Radics, Tetrahedron Lett., 1966, 4753.
- 2) G. Kusano, T. Takemoto, N. Aimi, H. J. C. Yeh, and D. F. Johnson, Heterocycles, 1975, 3, 697.
- 3) W. Gaffield, M. Benson, W.F. Haddon, and R. E. Lundin, Aust. J. Chem., 1983, 36, 325.
- 4) K. Schreiber, A. Walther, and H. Ronsch, Tetrahedron, 1964, 20, 1939.
- 5) O. Kennard, L. R. di Sanseverino, and J. S. Rollett, J. Chem. Soc. (C), 1967, 956.
- 6) W. Gaffield and R. F. Keeler, Adv. Exp. Med. Biol., 1984, 177, 241.
- 7) D. Brown and R. F. Keeler, J. Agric. Food Chem., 1978, 26, 564.

Table IV. Positional ($\times 10^4$) and Thermal Parameters (\AA^2) of II for Non-hydrogen Atoms with Their Standard Deviations in Parentheses.

Atom	x	y	z	Req.
C1	8683 (2)	3284(6)	4696(7)	5.9(3)
C2	9117 (1)	3366(6)	4925(8)	5.7(2)
C3	9195 (1)	4123(5)	6600(7)	5.5(2)
C3'	9749 (2)	5325(6)	7346(7)	6.1(3)
C4	10185 (2)	5340(1)	7310(1)	9.5(4)
C4	9024 (1)	3458(6)	8249(6)	5.5(2)
C5	8609 (1)	3278(5)	8044(6)	4.8(2)
C6	8362 (1)	3627(5)	9331(7)	5.5(2)
C7	7945 (1)	3508(6)	9285(7)	5.7(2)
C8	7803 (1)	2670(4)	7706(6)	4.3(2)
C9	8040 (1)	2918(5)	6013(6)	4.3(2)
C10	8469 (1)	2649(4)	6273(6)	4.5(2)
C11	7875 (1)	2219(6)	4380(6)	5.6(2)
C12	7448 (1)	2466(5)	4057(7)	5.5(2)
C13	7214 (1)	2132(5)	5749(6)	4.2(2)
C14	7385 (1)	2911(5)	7334(6)	4.4(2)
C15	7092 (1)	2722(6)	8835(7)	5.5(2)
C16	6721 (1)	2937(6)	7779(6)	5.3(2)
C17	6802 (1)	2685(5)	5772(6)	4.4(2)
C18	7227 (1)	680(5)	6082(9)	6.0(3)
C19	8555 (1)	1194(6)	6350(9)	6.3(3)
C20	6476 (1)	1781(5)	5162(6)	4.9(2)
C21	6326 (2)	2120(6)	3284(7)	6.0(2)
C22	6187 (1)	1902(5)	6720(7)	4.9(2)
C23	5922 (1)	3081(5)	6543(7)	5.4(2)
C24	5668 (2)	3201(6)	8177(8)	6.3(3)
C25	5445 (2)	1993(7)	8563(9)	6.9(3)
C26	5705 (2)	843(7)	8714(7)	5.4(3)
C27	5112 (2)	1805(9)	7170(1)	9.9(5)
N28	5959 (1)	756(4)	7049(6)	5.3(2)
C29	5952 (2)	-327(5)	6171(8)	6.2(3)
O1	9606.7(9)	4229(4)	6807(6)	7.0(2)
O2	9567 (1)	6277(6)	7754(7)	9.2(5)
O3	6430.5(8)	2035(3)	8255(4)	5.0(1)
O4	5759 (1)	-1276(5)	6447(6)	8.7(3)

- 8) D. Brown, "Structure - activity relationship of steroidal amine teratogens", Academic Press, New York, 1978, p. 154.
- 9) B. G. Tan, Y. L. Lam, H. H. Huang, and L. H. Chiba, J. Chem. Soc., Perkin Trans. 2, 1990, 2031.
- 10) G. Kusano, N. Aimi, and Y. Sato, J. Org. Chem., 1970, 35, 2624.

Received, 8th June, 1992