SPIROCYCLIC NORTRITERPENES FROM BULBS OF *SCILLA SCILLOIDES* II.[1]

NEW SPIROCYCLIC FURANOID NORTRITERPENES AND RELATED TETRANORTRITERPENE SPIROLACTONES

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Abstract ----- Five kinds of compounds, SSG-1 ~ -5, were isolated, besides 15-deoxoeucosterol (I), from fresh bulbs of *Scilla scilloides*. They were characterized respectively as 22β-hydroxy (III), 30-hydroxy (IV), 3-dehydro (V), 24,25,26-trinor-23-oxo (VI) and 24,25,26-trinor-22β-hydroxy-23-oxo (VII) derivatives of I on the basis of spectral data and by X-ray analysis for III. Although IV was identical with the compound reported by Parrilli et al, III and V are new natural products related to eucosterol (II) which was first isolated from *Eucomis* spp. and identified by Tamm et al. VI and VII are novel type of tetranortriterpene spiro-γ-lactones, and their coexistence with I and III is noted.

In the preceding paper[2] of this series it was reported that a free nortriterpene obtained from Me_2CO extracts of air-dried bulbs of *Scilla scilloides* Druce (Liliaceae) was characterized as 15-deoxo derivative (I) of eucosterol ((23*S*)-17,23-epoxy-3β,31-dihydroxy-27-nor-5α-lanost-8-ene-15,24-dione) (II), which was isolated from several *Eucomis* species and identified as such by Tamm et al[3] and later obtained together with three related compounds from *Muscari comosum* Mill. by Parrilli et al.[4] I was also suggested[2] to be contained in the form of its glycosides.

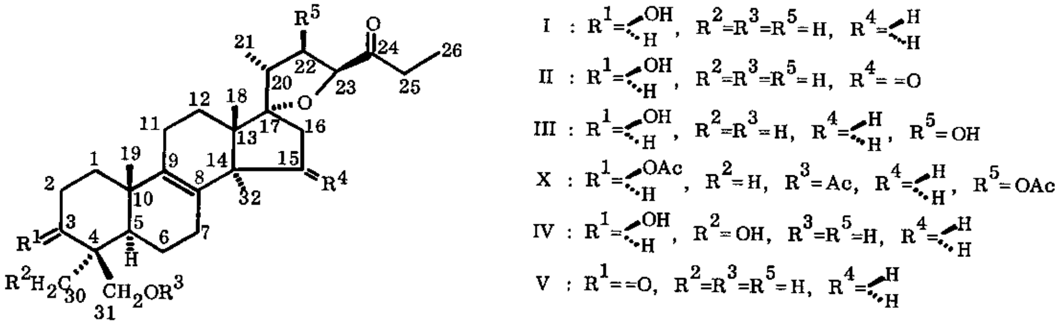
For the purpose of surveying analogs or homologs of I and their glycosides, the fresh bulbs (7 5 kg) collected in the suburbs of Fukuoka city during September were extracted with MeOH and examined. This paper concerns structural elucidation of five compounds so far isolated together with I.

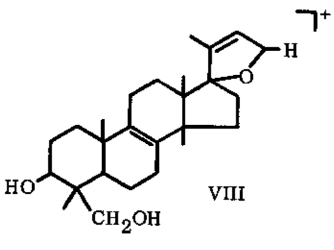
MeOH was removed from the extracts, the resulting aqueous solution shaken with AcOEt, the organic layer evaporated, and the residue was subjected to column chromatography successively on Sephadex LH-20, Diaion HP20AG and silica gel to give I (268 mg), compound SSG-1 (III) (51 mg), -2

Dedicated to Dr. Kyosuke Tsuda, Emeritus Professor of University of Tokyo, on his 75th birthday.

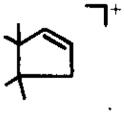
(IV) (9.4 mg), -3 (V) (9.0 mg), -4 (VI) (7.0 mg) and -5 (VII) (4.5 mg).

SSG-1 (III), colorless needles (from MeOH), mp 235-239°, $[\alpha]_D$-36.7°(c=0.22),[5] was slightly more polar than I and gave a similar purple color reaction with anisaldehyde / H_2SO_4[2] on silica gel TLC. It analyzed for $C_{29}H_{46}O_5$ and the ir[5] (3500 cm^{-1}(OH), 1705(isolated ketone)) and the electron impact mass (MS) spectra (m/z 474.333 ($M^{+\cdot}$) (calcd. for $C_{29}H_{46}O_5$, 474.335), 459 (M-CH_3), 441 (M-CH_3-H_2O), 399.291 (M-CH_3CH_2CO-H_2O) (calcd. for $C_{26}H_{39}O_3$ (VIII), 399.290), 329.246 (calcd. for $C_{22}H_{33}O_2$ (IX), 329.248), 57 ($CH_3CH_2CO^{\urcorner+}$)) suggested, in comparison with those[2] of I, that III is a congener of I bearing one hydroxy group in the side-chain at C_{17}. The ^1H nmr (pmr) spectral data of III and its triacetate (X), needles (from dil. MeOH), mp 201-204° (MS m/z: 600 ($M^{+\cdot}$)), were quite similar to those of I and its diacetate, respectively, except for the signals due to protons at C_{22} and C_{23} (Table I). From the above results and by comparison of the ^{13}C nmr (cmr) datum of III with that of I (Table II), III was presumed to have an additional hydroxy group at C_{22} of I in β-configuration.[6] In order to prove the presumed structure, X was then subjected to the X-ray





VIII



IX

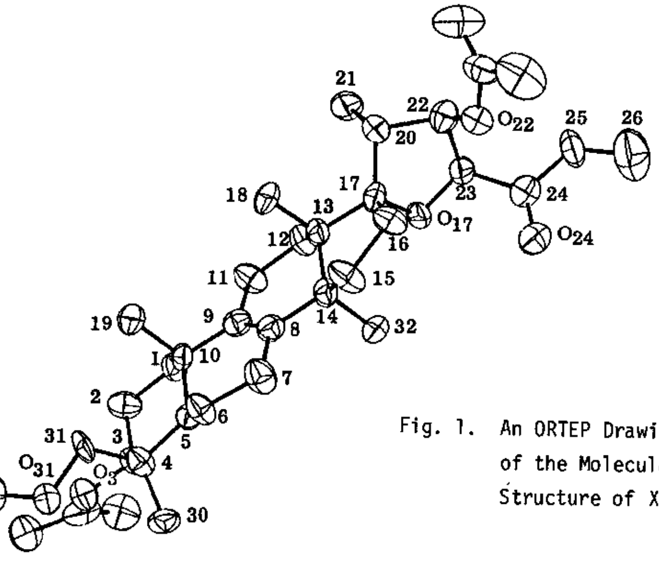


Fig. 1. An ORTEP Drawing of the Molecular Structure of X

diffraction study. A single crystal of X, $C_{35}H_{52}O_8$ (M.W., 600.80), suitable for analysis was obtained by recrystallization from isopropyl ether, and its data were as follows: size, 0.05 × 0.20 × 0.50 mm; monoclinic, space group $P2_1$ (Z=2); cell dimensions, a=19.178(9), b=7.585(3), c=11.824(5) Å, β=107.91(4)°, V=1636(1) $Å^3$; D(calcd.)=1.219 g/cm^3, D(obsd.)=1.200 (by flotation in KI solution). Of the 2793 reflections (by ω-scan method, $\omega < 25.0°$) collected on a Syntex $P\bar{1}$ automated diffractometer using graphite monochromated Mo-Kα (λ=0.71069 Å) radiation, 1991 were judged observed ($I > 2.0\ \sigma$). The structure was solved by the direct method (MULTAN 78)[7] and the atomic parameters except for the hydrogen atoms were refined by the block-diagonal least-squares of UNICS II[8] to a R-value of 0.092. A computer-generated perspective ORTEP[9] drawing of X (less hydrogen atoms) is shown in Fig. 1. Taking into account coexistence of III with I and VII described later, the absolute stereostructure of X was regarded to be the normal steroid type, that is, not the antipode of the figure.

Thus, III is 22β-hydroxy-15-deoxoeucosterol.

SSG-2 (IV) was obtained as colorless needles (from MeOH), mp 170-175°, $[\alpha]_D$-24.7° (c=0.40), $C_{29}H_{46}O_5$, positive reaction with anisaldehyde / H_2SO_4. The MS spectrum exhibited peaks at m/z 474.335 ($M^{+\cdot}$) (calcd., 474.335), 459 ($M-CH_3$), 417.301 ($M-CH_3CH_2CO$) (calcd. for $C_{26}H_{41}O_4$, 417.300), 345.247 ($C_{22}H_{33}O_3$) (calcd., 345.243), and 57 ($CH_3CH_2CO^{\urcorner +}$). The peaks at m/z 474, 459 and 57 were equally observed in that of III, but the other two, m/z 417 and 345, are larger in mass number than those at 399 and 329 yielded from III, respectively, by 18 (2H + O) and 16 (O), suggesting that IV is a positional isomer of III and has the additional hydroxy group not in the side-chain but in the A-D ring moiety of I. Comparison of its pmr and cmr data with those of III and I (Tables I and II) indicated that IV has the hydroxy group attached at C_{30} of I.

Therefore, IV is regarded as 30-hydroxy-15-deoxoeucosterol which had been isolated from bulbs of Muscari comosum and identified[10] by Parrilli et al.[4]

SSG-3 (V), colorless needles (from MeOH), mp 214-216°, $[\alpha]_D$-47.4° (c=0.93), $C_{29}H_{44}O_4$, showed in the ir spectrum hydroxy (3410 cm^{-1}) and two isolated ketone (1728, 1700) absorptions, and in the MS peaks at m/z 456.324 ($M^{+\cdot}$) (calcd., 456.324), 441 ($M-CH_3$), 399.288 ($M-CH_3CH_2CO$) (calcd. for $C_{26}H_{39}O_3$, 399.290), 327.230 (calcd. for $C_{22}H_{31}O_2$, 327.232) and 57 ($CH_3CH_2CO^{\urcorner +}$). From these and its pmr and cmr data in comparison with those of I (Tables I and II), V seemed to be the 3-dehydro derivative of I, and the structure was confirmed by identification of V monoacetate, mp 175-178°, with an authentic sample[2] derived from I.

Accordingly, V is 3-dehydro-15-deoxoeucosterol.

SSG-4 (VI) was obtained as colorless needles (from dil. MeOH), mp > 300°, $[\alpha]_D$+13.3° (c=0.54), $C_{26}H_{40}O_4$. IR: 3250 cm^{-1} (OH), 1765 (γ-lactone), no isolated ketone. MS m/z: 416.292 ($M^{+\cdot}$) (calcd., 416.293), 401 ($M-CH_3$), 329.246 (calcd. for $C_{22}H_{33}O_2$ (IX), 329.248), no fragment at 57. The above data, in particular, presence of the fragment IX which was also provided in the MS of I[2] and III,

Table I. PMR Spectral Data (100 MHz; δ in ppm; in $CDCl_3$ solution (* in py-d_5))

	I^2	I-diAc^2	III	III*	X	IV	V	VI	VII*
3-H^a	3.46	4.56	3.44	3.70	4.58	3.70		3.46	3.66
18-H_3^b	0.90	0.92	0.92	1.02	0.90	0.89	0.91	0.93	0.98
19-H_3^b	0.96	1.03	0.95	1.07	0.93	0.95	1.04	0.93	1.03
21-H_3^c	1.07	1.07	1.06	1.10	1.04	1.05	1.05	1.12	1.19
22-H			4.25(d) J=4.5Hz	4.59(d) J=4.5Hz	5.17(d) J=5Hz				4.33(d) J=3.5Hz
23-H	4.51(dd) J=7,10Hz	4.53(dd) J=7,10Hz	4.57(d) J=5Hz	4.87(d) J=5Hz	4.72(d) J=6Hz	4.51(dd) J=8,10Hz	4.51(dd) J=8,10Hz		
25-H_2^e	2.57	2.58	2.65	2.77	2.51	2.55	2.55		
26-H_3^f	1.07	1.07	1.07	1.12	1.05	1.06	1.05		
30-H_3 (#$^3H_2$)	1.25(s)	1.04(s)	1.26(s)	1.56(s)	1.18(s)	3.72 4.15 }#g	1.27(s)	1.23(s)	1.56(s)
31-H_2^g	3.36 4.23	4.15 4.37	3.32 4.23	3.64 4.58	4.15 4.38	3.78 4.34	3.56 4.02	3.32 4.21	3.70 4.55
32-H_3^b	1.23	1.24	1.23	1.64	1.24	1.22	1.24	1.08	1.28

a, multiplet ($W_{1/2}$=16~18 Hz); b, singlet; c, doublet (J=7 Hz); e, quartet (J=7 Hz);
f, triplet (J=7~7.5 Hz); g, AB quartet (J=11 Hz)

suggested that V has the same partial structure as I with respect to the A-D ring moiety and has the γ-lactone ring attached at C_{17}. The pmr spectrum of VI showed no signal due to 23-H and CH_3CH_2CO group, but exhibited the four tertiary and one secondary CH_3 signals identical with those of 13-, 10-, 4-, 14-[11] and 20-CH_3 groups of I (Table I). The δ and J values of three protons adjacent to O-functions in the spectrum of VI were also same as those of 31-H_2 and 3α-H of I. No olefinic proton signal was observed as in the case of I, indicating the Δ^8 structure. In the cmr spectrum of VI, signals at 39.0 ppm (t) and 97.6 (s) were identical with those of C_{16} and C_{17} of I, while triplet at 29.9ppm (vicinal to carbonyl) and singlet at 176.4 (carbonyl) were regarded to correspond to the triplet at 36.8 and the doublet at 81.4 ascribed respectively to C_{22} and C_{23} of I. Taking

Table II. CMR Spectral Data (25.05 MHz; δ in ppm; in py-d_5 solution (* in $CDCl_3$))

Carbon No.	I	I*[2]	III	IV	V*	VI
1	35.7	35.4	35.9(t)	35.7(t)	34.7(t)[f]	35.7(t)
2	28.9	28.3	29.0(t)	28.7(t)	35.4(t)[f]	28.9(t)
3	79.8	80.8	79.9(d)	73.9(d)	220.1(s)	79.7(d)
4	43.1	42.8	43.1(s)	46.9(s)	51.0(s)	43.1(s)
5	51.5	51.3	52.3(d)	44.1(d)	51.7(d)	51.4(d)
6	18.9	18.3	19.0(t)	19.0(t)	18.9(t)	17.9(t)
7	26.8	26.5	26.9(t)	26.5(t)	25.9(t)	26.7(t)
8	134.4	134.2	134.5(s)	134.6(s)	132.2(s)	134.3(s)
9	134.9	135.2	135.2(s)	135.1(s)	135.8(s)	135.2(s)
10	37.0	36.8	37.1(s)	37.0(s)	36.7(s)	37.0(s)
11	21.0	20.8	21.2(t)	21.1(t)	20.8(t)	20.8(t)
12	25.2	24.9	25.4(t)	25.3(t)	24.8(t)	24.7(t)
13	48.7	48.6	50.0(s)	48.7(s)	48.5(s)	48.9(s)
14	50.7	50.6	50.3(s)	50.7(s)	50.6(s)	50.9(s)
15	31.9[a]	31.7	32.6(t)[b]	32.0(t)[c]	31.8(t)	31.5(t)
16	39.6	39.7	40.4(t)	39.6(t)	39.5(t)	39.0(t)
17	96.8	97.2	97.3(s)	96.9(s)	96.8(s)	97.6(s)
18	19.2	19.2	19.6(q)	19.3(q)	19.3(q)	18.8(q)
19	20.0	19.9	20.4(q)	19.9(q)	19.6(q)	20.0(q)
20	43.6	43.6	51.6(d)	43.6(d)	43.5(d)	41.7(d)
21	17.2	17.2	16.2(q)	17.2(q)	17.1(q)	17.6(q)
22	36.8	36.8	80.6(d)	36.8(t)	36.7(t)	29.9(t)
23	81.4	81.4	87.9(d)	81.4(d)	81.3(d)	176.4(s)
24	211.8	213.5	210.6(s)	211.9(s)	212.8(s)	
25	32.2[a]	32.3	33.7(t)[b]	32.2(t)[c]	32.2(t)	
26	7.7	7.5	7.6(q)	7.7(q)	7.4(q)	
30	23.3	22.3	23.3(q)	62.9(t)[e]	21.7(q)	23.4(q)
31	64.2	64.4	64.3(t)	63.3(t)[e]	65.6(t)	64.2(t)
32	26.2	25.8	26.6(q)	26.2(q)	26.1(q)	25.7(q)

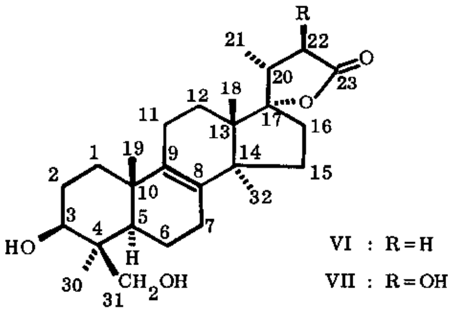
a, b, c, e, and f may be reversed on each vertical column.

all these data into account, VI was considered to be a 17-spiro-γ-lactone which corresponds to I, and the β-configuration of the side-chain at C_{17} and hence α-orientation of the C_{17}-O linkage, as in the case of I, was evidenced by the CD spectrum (c=0.09, MeOH): $\Delta_{\varepsilon}^{23}$ +0.31 (240) (positive max.).

Consequently, VI is defined as 24,25,26-trinor-23-oxo-15-deoxoeucosterol.

SSG-5 (VII), colorless needles (from MeOH), mp 285-289°, $[\alpha]_D$+19.4° (c=0.30), $C_{26}H_{40}O_5$, showed in the ir spectrum hydroxy (3400 cm^{-1}) and γ-lactone (1765) absorptions, and in the MS peaks at m/z 432.288 ($M^{+\cdot}$) (calcd., 432.288), 399.254 (M-CH_3-H_2O) (calcd. for $C_{25}H_{35}O_4$, 399.254), and 329.248

($M-C_4H_7O_3$) (calcd. for $C_{22}H_{33}O_2$ (IX), 329.248), but none at 57. In consideration of these data together with those of III and VI, and in analogy to the relationship between VI and I, VII was assumed to be the spiro-γ-lactone related to III and bearing a hydroxy group at C_{22} of VI. When the pmr spectrum of VII was compared with that of III, their similarity and difference were parallel to those between VI and I, and the doublet ($\underline{J}$=4.5 Hz) at 4.59 ppm attributed to 22α-H of III was replaced by a signal (d, $\underline{J}$=3.5 Hz) at 4.33 in the spectrum of VII. This new signal was assigned to 22-H of VII, and, on the basis of its chemical shift and $\underline{J}$ value which resembled that due to the coupling between 20β- and 22α-H of III, the proton was considered to be α-oriented, geminal to hydroxy and vicinal to carbonyl and methine groups. Thus, the assumed structure of VII was proved. Although VII showed, in contrast to VI, a negative Cotton curve in the CD spectrum ($\underline{c}$=0.03, MeOH, $\Delta_{\varepsilon}^{23}$ -1.62 (224) (negative max.)), since a strong negative contribution to the Cotton effect of β-hydroxy group vicinal to carbonyl function is known,[12] the absolute stereo-structure of VII could be safely regarded as same as that of VI.



VI : R=H

VII : R=OH

Now, it follows that VII is 24,25,26-trinor-22β-hydroxy-23-oxo-15-deoxoeucosterol.

III and V are new compounds congeneric to II, while VI and VII are novel type of tetranortriterpene spiro-γ-lactones closely related to I and III, respectively. Co-occurrence of I and III with VI and VII is particularly noteworthy from the view point of metabolism (or biosynthesis)[13] of a new series of natural products, spirocyclic furanoid nortriterpenes.

Acknowledgement ----- The authors are grateful to Assoc. Prof. H. Okabe of Fukuoka University for his kind help for collection and extraction of the plant material, and to Assoc. Prof. T. Komori of this laboratory, Prof. I. Ueda and Assoc. Prof. S. Kawano of College of General Education, Kyushu University, for the valuable discussions on X-ray analysis. Thanks are also due to Assoc. Prof. K. Mihashi and Mr. T. Nishi of Fukuoka University, to Prof. T. Kimura of Daiichi College of Pharmacy, to Mr. Y. Tanaka and Miss Y. Soeda of this Faculty, respectively, for high resolution mass, CD, cmr and pmr spectra. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged.

References and Footnotes

1. Part I: see ref. 2.
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5. Optical rotations were measured at 18 ~ 20°C in $CHCl_3$-MeOH (1:1, *v/v*) solution and ir spectra were taken in KBr disk.
6. The Dreiding model shows that the dihedral angle of 20β- and 22α-H is approx. 120° suggesting that their coupling constants in the pmr are *ca*. 4 Hz (M. Karplus, *J. Am. Chem. Soc.*, 1963, **85**, 2870).
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10. Mps of IV (170-175°) and its orthoacetate (195-197°(from *n*-hexane)) were different from those (amorphous, 168-170°(from hexane), respectively) given by Parrilli et al,[4c] but the pmr spectral data (at 100 MHz) of both compounds were in good agreement with reported values (at 270 MHz).[4c]
11. Only this signal showed a slight upfield shift (0.15 ppm).
12. G. Snatzke, H. Ripperger, C. Horstmann, and K. Schreiber, *Tetrahedron*, 1966, **22**, 3103.
13. Another metabolite, 3β-hydroxy-4β-hydroxymethyl-4,14α-dimethyl-15-oxo-24-nor-5α-chola-8,16-diene-23-oic acid, has been reported[3b] to co-occur with II and its 16β-hydroxy derivative.

Received, 28th August, 1981