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# SPIROSTANE DERIVATIVES HIGHLY OXYGENATED AT A AND B RINGS FROM THE RHIZOMES OF ROHDEA JAPONICA ROTH AND OF CAMPYLANDRA AURANTIACA BAKER

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Abstract: Two steroidal compounds BR-1 (I) and -2 (II) which had been obtained from the rhizomes of Campylandra aurantiaca Baker, were isolated also from those of Rohdea japonica Roth. They were identified, respectively, by X-ray analysis and chemical correlation as 1β,2β,3β,4β,5β,7α-hydroxy-spirost-25(27)-en-6-one and its corresponding 6β-hydroxy compound.

The rhizomes of Rohdea (Rhodea) japonica Roth (Liliaceae) have been known as a cardioactive crude drug, but none of their ingredients has so far been characterized.[1] In the meantime two complex steroidal compounds BR-1 (I) and -2 (II), the structures of which remain unknown due to their small amounts available, had been isolated in this laboratory from the rhizomes[2] of an East Himalayan plant Campylandra aurantiaca Baker which is regarded to be related to R.japonica. In expectation of the existence of I and II, and in order to clarify extensively the steroidal constituents, a chemical study on the rhizomes of R.japonica has been carried out. This communication is to report actual isolation of I and II from R.japonica and their structures which represent the novel spirostane derivatives highly oxygenated at A and B rings.

The MeOH extractives of the sliced fresh materials[3] were fractionated by the partition methods (water - BuOH, water - (BuOH + AcOEt)) and repeated column chromatographies over silica gel 60 ($CHCl_3$ - MeOH - water, MeCOEt - water) to give R-1 (I') and -2 (II') both being homogeneous and identical, respectively, with I and II on TLC using several different kinds of solvent systems.

R-1 (I'), a white powder (dil. MeOH), mp 240 - 241° (decomp.), $[\alpha]_D$ -98.0° (pyridine), $C_{27}H_{40}O_9$ ($M^+$, 508), shows on the IR spectrum (KBr disk) the characteristic absorptions[4] of spirost-25(27)-ene (980, 925, 882, 858 $cm^{-1}$) together with hydroxyl (3400) and six-membered ring ketone (1717). On usual acetylation I' afforded an acetate (III'), the PMR spectrum ($CDCl_3$) of which exhibits the signals of 18- (0.77 ppm), 19- (0.96) and 21-methyl (0.98) groups, and five acetoxyl (2.01 - 2.15) and one hydroxyl (3.77) protons. The above data suggest that I' is a spirost-25(27)-ene derivative having one carbonyl group at A, B or C ring, five acylable and one tertiary or hindered hydroxyl functions. However, the PMR spectrum of III' shows, besides a singlet (2H, 4.75 ppm) and a double doublet (2H, 3.48 and 4.26) respectively ascribable to the

terminal 27-methylene and the equatorial and axial protons at $C_{26}$, another double doublet (2H, 3.92 and 3.25) with relatively much lower intensity assignable to 26-methylene next to the 25-methine bearing axial (β) 27-methyl group. It indicates that III' and hence I' are accompanied by a minute amounts of the corresponding 25-L-spirostane derivatives. Subsequently, further purification of III' was tried and successfully achieved by HPLC (Waters Associates RCM-100) using Radial Pak A No. 01244 as a column and 86% MeOH (8 ml/min.) as an eluent to provide III", colorless needles (dil. MeOH), mp 177 - 180°, which was not any more contaminated with the 25-L-analog as indicated by its PMR spectrum. However, an attempted regeneration of pure R-1 (I") from III" with alkali was not successful and yielded a new compound (IV), colorless plates (MeOH), mp 166 - 169°, $C_{23}H_{32}O_4$ ($M^+$, 372); PMR ($CDCl_3$, ppm), 0.83 (18-methyl), 1.88 (19-methyl), 0.97 (21-methyl), 4.75 (27-methylene). Acetylation of IV gave an acetate (V), colorless needles (MeOH), mp 224 - 228°, $C_{25}H_{34}O_5$ ($M^+$, 414); IR (KBr disk, $cm^{-1}$), 1765 (acetoxyl), 1687, 1637 (enone), 957, 919, 895, 874 (spirost-25(27)-ene); PMR, 0.85 (18-methyl), 1.83 (19-methyl), 0.96 (21-methyl), 2.24 (acetoxyl) (no signal of the proton geminal to acetoxyl), 4.74 (27-methylene).

On the other hand, BR-1 (I), a white powder (dil. MeOH), mp 244 - 247° (decomp.), $[\alpha]_D$ -86.9° (pyridine), $C_{27}H_{40}O_9$ ($M^+$, 508), was acetylated as I' to give an acetate (III), colorless needles (dil. MeOH), mp 176 - 180°, which was identified with III" by mixed mp and by comparison of their PMR spectra. Therefore I is regarded to be identical with pure R-1 (I"), and in order to determine the molecular structure it was subjected to X-ray analysis.

Since a crystal of I (colorless prism from dil. EtOH) turned to a white powder on exposure to the air, the analysis was carried out by using the crystal enclosed together with the solvent in a sealed glass capillary. The crystal data were as follows: size, 0.2 × 0.2 × 0.8 mm; monoclinic space group $P2_1$ (Z=2); cell dimensions, $\underline{a}$=18.0954(37), $\underline{b}$=7.5287(14), $\underline{c}$=10.6818(28) Å, β=104.518(17)°, V=1408.75(51) $Å^3$; D (calcd.)=1.200, D (obsd.)=1.241 (by flotation in KI solution). Of the 4452 reflections (by θ - 2θ scanning, $2\theta \leq 60°$) collected on a Syntex $P\bar{1}$ automated diffractometer using graphite monochromated Mo Kα (λ=0.71069 Å) radiation, 2715 were judged observed ($I \geq 2.3\sigma(I)$). The structure was solved by direct methods.[5] The atomic parameters except for the hydrogen atoms of hydroxyl groups were refined by the block-diagonal least-squares to a R-value of 0.067. A computer-generated perspective drawing of I (less hydrogens) is given in Fig. 1 and it represents the absolute configuration of I, because I' shows a negative Cotton curve ($[\phi]^{276nm}_{peak}$ 6770, $[\phi]^{319nm}_{trough}$ -9040, $\underline{a}$= -158) on the ORD spectrum.

Consequently I (=I") is designated as 1β,2β,3β,4β,5β,7α-hexahydroxy-spirost-25(27)-en-6-one. A new compound IV yielded by saponification of III" is presumed on the basis of its molecular formula and spectral data as 5-hydroxy-1,2,3,4-tetranor-spirost-5(10),25(27)-dien-6-one, and Scheme 1 is tentatively proposed as a probable degradation pathway.[6]

R-2 (II'), colorless needles (dil. MeOH), mp 273 - 275°, $[\alpha]_D$ -98.5° (pyridine), $C_{27}H_{42}O_9$ ($M^+$, 510), shows on the IR spectrum (KBr) the hydroxyl (3700 - 3000 $cm^{-1}$), the $\Delta^{25(27)}$-spiroketal (979, 925, 880, 850) but no carbonyl absorptions. Usual acetylation gave an acetate (VI'), colorless needles (dil. MeOH), mp 176 - 178°, which exhibits on the PMR spectrum ($CDCl_3$) the signals of six acetoxyl groups, one hydroxyl proton and 26-methylene only next to the olefinic function at $C_{25(27)}$. These data suggest that II' is a heptahydroxy-spirost-25(27)-ene corresponding to I. Although the $NaBH_4$ reduction of I' in MeOH provided a product much more polar than II' on TLC,

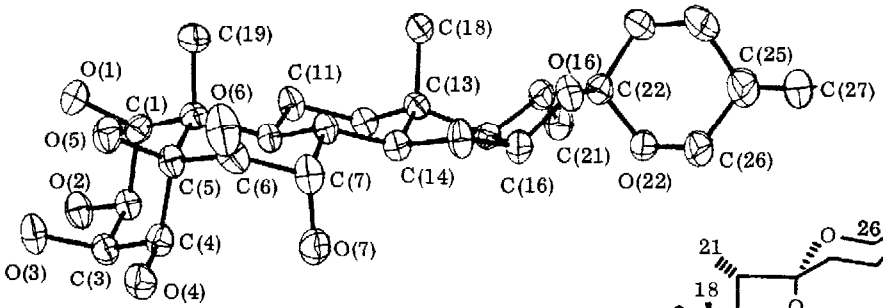
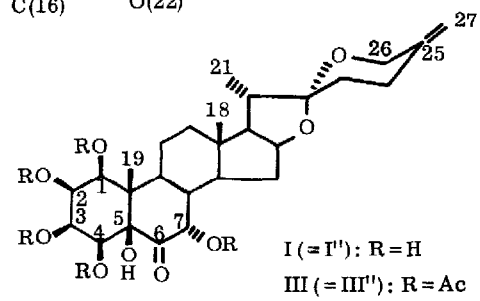
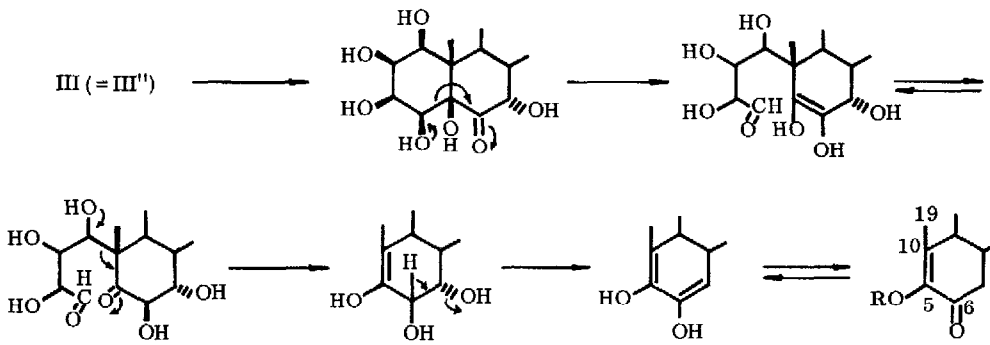



Fig. 1



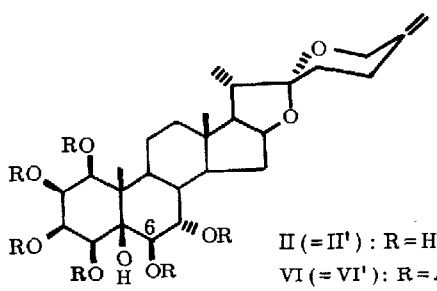

I (= I'') : R = H
III (= III'') : R = Ac



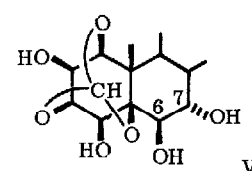

Scheme 1




IV : R = H
V : R = Ac




II (= II') : R = H
VI (= VI') : R = Ac




VII

a similar reduction of the acetate III' followed by reacetylation gave an acetate (VI"), mp 174 - 178°, having the same Rf value as that of VI'. The PMR spectrum of VI" was identical with that of VI' except for the weak signal (2H, double doublet, at 3.92 and 3.25 ppm) due to 26-methylene of the 25-L-spirostane analog contaminated in VI". Therefore II' is considered to be a pure hepta-hydroxy compound corresponding to I (=I"). It is generally accepted[7] that the metal hydride reduction of the carbonyl function at $C_6$ of a steroid having 19-methyl group affords predominantly (>90%) 6β-hydroxy compound. Furthermore, the orthoester (VII), mp >300°, derived from II' was neither oxidized with $NaIO_4$ nor derivatized to an acetonide indicating the trans-diaxial orientation of the two hydroxyl groups at $C_6$ and $C_7$.

Accordingly it could be safely concluded that II' is 1β,2β,3β,4β,5β,6β,7α-heptahydroxy-spirost-25(27)-ene.

BR-2 (II), colorless needles (dil. MeOH), mp 273 - 275.5°, $[\alpha]_D$ -95.5° (pyridine), and its acetate (VI), colorless needles (dil. MeOH), mp 176 - 178°, were identified respectively with II" and VI' by mixed mp and by comparisons of their IR and PMR spectra.

I (=I") and II (=II') are noteworthy as the first reported natural spirostane derivatives highly oxygenated at A and B rings.[8]

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## References and Notes

1) A cardiac glycoside "rhodein" has been reported (T.Murashima, Tohoku J.Exp.Med., 8, 405 (1927)), but the structure is unknown.

2) The authors thank Prof. O.Tanaka of Hiroshima University for the material.

3) Collected in Fukuoka, Miyazaki, and Kyoto Prefectures. Our thanks are due to Prof. I.Nishioka of this University, Dr. H.Okabe of Fukuoka University, Dr. M.Goto and Dr.T.Matsuoka of Takeda Chemical Industries, Ltd. for supplying the rhizomes.

4) R.Tschesche, H.Schwarz, and G.Snatzke, Ber., 94, 1699 (1961).

5) G.Germain, P.Main, and M.M.Woolfson, Acta Crystallogr., A27, 368 (1971).

6) The valuable suggestions by Prof. K.Kanematsu and Dr. K.Fujita of this University are gratefully acknowledged.

7) D.N.Kirk and M.P.Hartshorn, "Steroid Reaction Mechanisms," Elsevier Pub.Co., Amsterdam, 1968, p. 136.

8) Takahira and his collaborators reported isolation of a steroid presumed to be 1,2,3,4,5,6-hexahydroxy-spirost-25(27)-en-7-one together with a series of 3α-hydroxy-spirostane derivatives from a kind of crude drug "Senshoku-shichikon," but the structure, particularly the stereochemistry, remains unestablished (M.Takahira, Y.Kondo, G.Kusano, and S.Nozoe, Abstracts of Papers, the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978, p. 337).

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