Comparison with colorimetric methods based on the enzymatic reaction (CEH–COD–POD system) with chromogenic substrates (4-aminoantipyrine-phenol[2,11]) for serum total cholesterol and HDL cholesterol showed correlation coefficients of 0.933 ($n=48$) and 0.975 ($n=30$), respectively, and the regression equations for the present method (x) against the colorimetric methods were $y=1.03x+2.2$ and $y=0.98x+2.2$, respectively. This indicates that the present method gives values virtually identical to those obtained by the colorimetric methods.

The fluorimetric method is precise and simple, and should be useful in cases where only an extremely small amount of serum is obtainable.

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4,4-Dimethyl Effect. (1). Stereochemistries of the Hydrogenation Products of α-Onocerin[1,2]

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Three isomeric hydrogenation products of α-onocerin diacetate, onoceranediol diacetates-I, -II, and -III, were converted to the corresponding diones-I, -II, and -III, which exhibited a positive, a double humped, and a negative CD spectrum, respectively. The configurations of onocerane-I, -II, and -III were therefore elucidated as 8αH-14βH, 8αH-14αH, and 8βH-14αH, respectively.

Keywords——α-onocerin; onoceranes; onocerane-3,21-diones; CD spectra; 4,4-dimethyl effect; 4,4,10-trimethyl-*trans*-decalin-3-ones

The structure and stereochemistry of α-onocerin (**1**), a tetracyclic triterpenoid of *Ononis spinosa* (Leguminosae) were determined more than two decades ago.[4,5] Later, this triterpenoid was found in various plants of *Lycopodium* (Lycopodiaceae) as a common constituent and it is regarded as a probable biogenetic precursor of the triterpenoids of the serratane group.[6] During its structure investigation, Barton and Overton[4] prepared three isomeric hydrogenation products, onoceranediol-I, -II, and -III. The stereochemistries of these compounds, however, were not determined until we correctly assigned their configurations as **3**, **4**, and **5**, respectively, in 1975.[2] This paper presents details of the experiments.

Hydrogenation of α-onocerin diacetate (**2**) in EtOAc over PtO_2 produced, in accord with the previous result,[4] three isomeric diacetates which were separated by column chromatography on acid-washed alumina. Alkaline hydrolysis of the diacetates-I, -II, and -III, and Jones oxidation of the resulting diols afforded the diketones-I, -II, and -III, respectively. The mp's of all these derivatives are in accord with the data reported previously.[4]

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1) Triterpenoid Chemistry. XIV. Part XIII: T. Sano, T. Fujimoto, and Y. Tsuda, *Chem. Pharm. Bull.*, **23**, 1784 (1975).
2) A preliminary report of this work was presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan. Y. Tsuda, T. Sano, and T. Fujimoto, Abstract-II, p. 253, Nishinomiya, April (1975).
3) Location: *a) 13-1 Takara-machi, Kanazawa 920, Japan*; *b) 5-1-8 Tsurumaki, Setagaya-ku, Tokyo 154, Japan.*
4) D.H.R. Barton and K.H. Overton, *J. Chem. Soc.*, **1955**, 2639.

The NMR spectra of these compounds were almost useless for elucidation of their configurations, since all the methyl groups appeared in a very narrow range.

The CD spectra of onoceranediones-I, -II, and -III provided important information on their configurations. Interestingly, they were all profoundly different. Onoceranedione-I exhibited a curve with a positive Cotton effect at 289 nm ($\Delta\varepsilon$= +1.50), whereas onoceranedione-III exhibited a curve with a negative Cotton effect at 302 nm ($\Delta\varepsilon$=−0.902). The latter curve was identical in shape and λ_{max} with those of 4,4-dimethylsteroid 3-ketones, and the former was similar to those of 4,4,8-trimethylsteroid 3-ketones (or triterpenoid 3-ketones).[7] In contrast, onoceranedione-II showed a typical double humped CD curve which was found to be identical with the aristhmetic sum of the spectra of the dione-I and the dione-III [$\Delta\varepsilon_{II}=1/2(\Delta\varepsilon_{I}+\Delta\varepsilon_{III})$], indicating that a half of dione-II is identical with dione-I and the other half is identical with dione -III (see Figure).

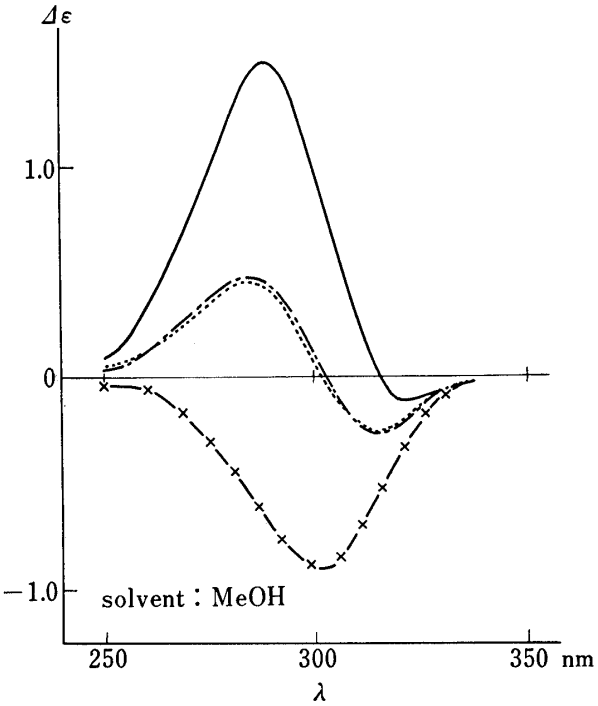

Fig. 1. Observed and Calculated CD Spectra of Onoceranedione-I, II, and -III

——————: dione-I (**9**)
——×——: dione-III (**11**)
——·——·: dione-II (**10**), obs.
----------: dione-II (**10**), calcd. [$\Delta\varepsilon=1/2(\Delta\varepsilon_{I}+\Delta\varepsilon_{III})$]

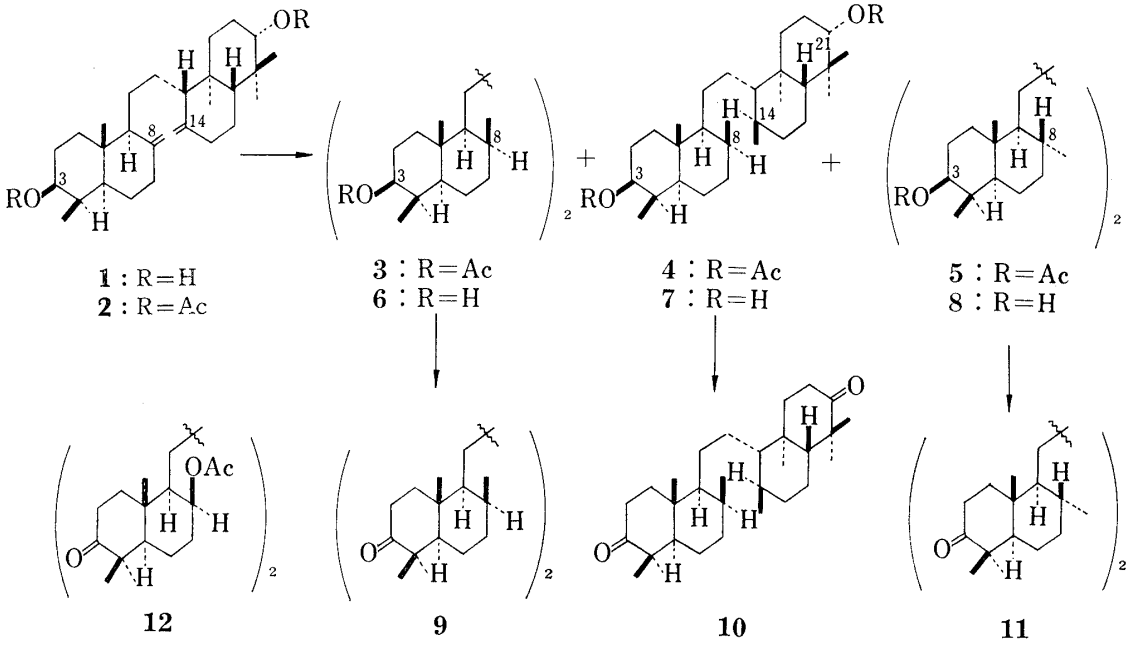

Chart 1

5) K. Scheffer, R. Vitro, D. Arigoni, and O. Jeger, *Helv. Chim. Acta*, **39**, 174 (1956).
6) Y. Tsuda, T. Fujimoto, K. Isobe, T. Sano, and M. Kobayashi, *Yakugaku Zasshi*, **94**, 970 (1974).
7) P. Witz, H. Herrmann, J-M. Lehn, and G. Ourisson, *Bull. Soc. Chim. Fr.*, **1963**, 1101.

These results, when coupled with the finding that 8β, 14α-diacetoxy-26,27-bisnoronocerane-3,21-dione (**12**)[8] has a positive Cotton effect at 280 nm ($\Delta\varepsilon=+0.96$) in methanol led to the conclusion that onoceranedione-I had an 8αH (and 14βH) configutraion (**9**), like that of triterpenoid 3-ones, and onoceranedione-III had an 8βH (and 14αH) configuration (**11**), like that of usual 4,4-dimethylsteroids. Accordingly, onoceranedione-II is 8αH,14αH-onocerane-3,21-dione (**10**). Apparently, the anomaly of the "4,4-dimethyl effect"[9] in 4,4-dimethylsteroid-3-ketones is copied in a bicyclic system such as onoceranoid: the Cotton effect is inverted from negative to positive by introduction of the 8β-methyl group.

Besides triterpenoids related to onoceranoids, there are various terpenoids, such as labdanoids and drimanoids, possessing an 8,9-disubstituted-4,4,10-trimethyl-*trans*-decalin system. Some of them occur in both enantiomeric forms: *i.e.* labdanoids and *ent*-labdanoids. Care should therefore be exercised in the determination of their absolute stereochemistry from the CD (or ORD) spectra of the corresponding 3-ketones, since the sign of the Cotton effect could be reversed, depending on the stereochemistry of the C_8-substituent.

The changes of the Cotton effect in relation to conformational changes of ring A in terpenoids and the solvent effect will be fully discussed in a forthcoming publication.

Experimental

Unless otherwise stated, mp's were taken on a Yanagimoto micro hot-stage mp apparatus, and are uncorrected. IR spectra were taken in Nujol mulls using a Hitachi-214 spectrometer and are given in cm^{-1}. NMR spectra were recorded in $CDCl_3$ solutions on a Varian T-60 (60 MHz) or a JEOL FX-100 (100 MHz) spectrometer with TMS as an internal reference. CD spectra were taken in methanol solutions using a Jasco J-20 spectrometer; concentrations are given in g/ml.

Hydrogenation of α-Onocerin Diacetate (**2**)——α-Onocerin diacetate **2** (2 g) in EtOAc (200 ml) was hydrogenated over PtO_2 (500 mg) for 10 hr, as described previously.[4] Chromatography of the product in benzene on acid-washed alumina and several crystallizations of the eluates gave onoceranediol-III diacetate (100 mg), onoceranediol-II diacetate (50 mg), and onoceranediol-I diacetate (150 mg), in that order of elution.

Onoceranediol-I Diacetate (**3**): Needles from benzene–methanol, mp 228—229° (lit. mp 216—218°[4]). NMR (60 MHz) δ: C-Me 0.83 (21H), 0.92 (3H, half of a doublet); OAc 2.00 (6H); >C<u>H</u>–OAc 4.43 (2H, m).

Onoceranediol-II Diacetate (**4**): Prisms from CH_2Cl_2–methanol, mp 173—174° (lit. mp 171—172°[4]). NMR (60 MHz) δ: C-Me 0.80 (1.5H, half of a doublet), 0.85 (21H), 0.93 (1.5H, half of a doublet), OAc 2.01 (6H); >C<u>H</u>–OAc 4.42 (2H, m).

Onoceranediol-III Diacetate (**5**): Needles from methanol, mp 149—150° (initially melted at 124—125° then resolidified) (lit. mp 150—151°[4]). NMR (60 MHz) δ: C–Me 0.80 (6H), 0.85 (15H), 0.92 (3H, half of a doublet); OAc 2.03 (6H); >C<u>H</u>–OAc 4.43 (2H, m).

Alkaline Hydrolysis and Jones Oxidation of Onoceranediol Diacetates——Diacetate-I, -II, and -III (50—100 mg) in 5% methanolic KOH (10 ml) were each heated under reflux for 2 hr. Concentration of the mixtures and addition of water provided precipitates which were collected by filtration. They showed no CO absorption in the IR spectra. The resulting diol-I (mp 259—263°), -II (mp 176—177°), -III (mp 200—201°) (20—50 mg) in acetone (5—10 ml) were each oxidized with Jones reagent (a few drops) at 0° for 15 min. The mixture was diluted with water and extracted with ether. Concentrations of the dried extract gave the dione-I, -II, and -III, respectively.

Onoceranedione-I (**9**): Prisms from CH_2Cl_2–methanol, mp 212—213° (lit. mp 212—213°[4]) IR: 1715. NMR (100 MHz) δ: –C(–)–Me 0.95, 1.04, 1.09 (each 6H, s); C_8- and C_{14}–Me 0.94 (6H, d, $J=7.6$ Hz). CD ($c=0.336\times10^{-3}$) $\Delta\varepsilon$ (nm): −0.06 (330), −0.08 (325), −0.114 (320), −0.12 (319), 0 (315), 0.22 (310), 0.56 (305), 0.96 (300), 1.32 (295), 1.48 (290), 1.50 (289), 1.43 (285), 1.28 (280), 0.98 (275), 0.76 (270), 0.51 (265), 0.32 (260), 0.16 (255), 0.08 (250).

Onoceranedione-II (**10**): Prisms from CH_2Cl_2–methanol, mp 164—165° (lit. mp 160—162°[4]). IR: 1713. NMR (100 MHz) δ: C–Me 0.92, 0.97, 1.03, 1.06, 1.08 (total 24H). CD ($c=0.927\times10^{-3}$) $\Delta\varepsilon$ (nm): −0.014 (340), −0.033 (335), −0.072 (330), −0.13 (325), −0.23 (320), −0.29 (315), −0.25 (310), −0.11 (305), 0 (302), 0.095 (300), 0.290 (295), 0.42 (290), 0.470 (285), 0.473 (283), 0.455 (280), 0.38 (275), 0.29 (270), 0.21 (265), 0.13 (260), 0.076 (255), 0.043 (280).

Onoceranedione-III (**11**): Prisms from methanol, mp 174—175° (lit. mp 168—169°[4]). IR: 1705. NMR (100 MHz) δ: –C(–)–Me 0.96, 1.02, 1.05 (each 6H, s); C_8– and C_{14}–Me 0.92 (6H, d, $J=5.7$ Hz). CD ($c=$

8) T. Sano, T. Fujimoto, and Y. Tsuda, *Chem. Pharm. Bull.*, **23**, 1784 (1975).
9) As leading references regarding this effect, see ref. 7) and C. Djerrassi, O. Halpern, and B. Riniker, *J. Am. Chem. Soc.*, **80**, 4001 (1958).

0.604×10^{-3}) $\Delta\varepsilon$ (nm): −0.033 (335), −0.066 (330), −0.165 (325), −0.325 (320), −0.630 (315), −0.688 (310), −0.847 (305), −0.902 (302), −0.891 (300), −0.858 (295), −0.731 (290), −0.583 (285), −0.429 (280), −0.313 (275), −0.198 (270), −0.116 (265), −0.044 (260), −0.033 (255), −0.022 (250).

8β,14α-Diacetoxy-26,27-bisnoronocerane-3,21-dione (12)[8]——CD ($c=0.174 \times 10^{-3}$) $\Delta\varepsilon$ (nm): −0.11 (320), −0.18 (315), −0.18 (311), 0 (309), 0.17 (300), 0.48 (295), 0.70 (290), 0.85 (285), 0.96 (280), 0.92 (275), 0.79 (270), 0.61 (265), 0.46 (260), 0.42 (255), 0.26 (250), 0.18 (245), 0.13 (240).

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Chemische und chemotaxonomische Untersuchungen von Filices. XXXII.[1] Chemische Untersuchungen der Inhaltsstoffe von *Plenasium banksiifolium* (Pr.) Pr.

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The fronds of *Plenasium banksiifolium* (Pr.) Pr. contain stigmastan-3β,5α-diol-6-one and its C_{28}-homolog, which are previously unreported in nature, together with the known compounds, ecdysone, ecdysterone, astragalin, stigmastan-3β,5α,6β-triol and its C_{28}-homolog.

Keywords——*Plenasium banksiifolium*; fern; chemotaxonomy; phytoecdysones; flavonol-glucoside; phytosterols; GLC-MS-coupling

Nach H. Hikino und Mitarb. besitzt *Plenasium banksiifolium* (Pr.) Pr. (jap. Name: Shiroyama-zenmai) eine starke Häutungshormonaktivität.[3] In Fortsetzung unserer chemischen und chemotaxonomischen Untersuchungen von Filices wurde *P. banksiifolium* auf die Inhaltsstoffe untersucht. Die oberirdischen Teile enthalten neben Ecdyson,[4] Ecdysteron,[5] und Astragalin (Kaempferol 3-O-β-D-glukosid)[6] zwei weitere Sterine I und II, die nur als Homologen-Gemische isoliert wurden.

I, Schmp. 242—245°, wurde durch den Vergleich mit dem synthetischen Produkt aus β-Sitosterin (begleitet von 10% Campesterin) als Stigmastan-3β,5α,6β-triol (III)[7] mit Beimengung des C_{28}-Homologen (20%) identifiziert.

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2) Adresse: *a) Funakawara-machi, Shinjuku-ku, Tokyo, 162, Japan*; *b) Kita 12, Nishi 12, Kita-ku, Sapporo, 060, Japan*; *c) Arise, Igawatani-machi, Tarumi-ku, Kobe, 673, Japan*; *d) Kuang Fu Road, Hsinchu, Taiwan, China.*

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