Tetrahedron. Vol. 24, pp. 6535 to 6541. Pergamon Press 1968. Printed in Great Britain

STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—LVII[1]

THE STRUCTURE OF IGAGENIN

F. YASUDA, Y. NAKAGAWA, A. AKAHORI and T. OKANISHI

Shionogi Research Laboratory, Shionogi & Co. Ltd., Fukushima-ku, Osaka, Japan

(*Received in Japan* 30 *April* 1968; *Received in the UK for publication* 24 *June* 1968)

Abstract—IR, NMR and mas spectra of igagenin isolated from *Dioscorea tokoro* Makino established that one of the three OH groups is at C_{27}. Elimination of this OH group by tosylation and subsequent LAH reduction afforded yonogenin. The structure of igagenin was confirmed as 25D, 5β-spirostane-2β, 3α, 27-triol.

THE steroidal sapogenins isolated from *Dioscorea tokoro* Makino, except diosgenin, have extraordinary characteristics.[2-7] They have an α-OH group at C_3 and exist

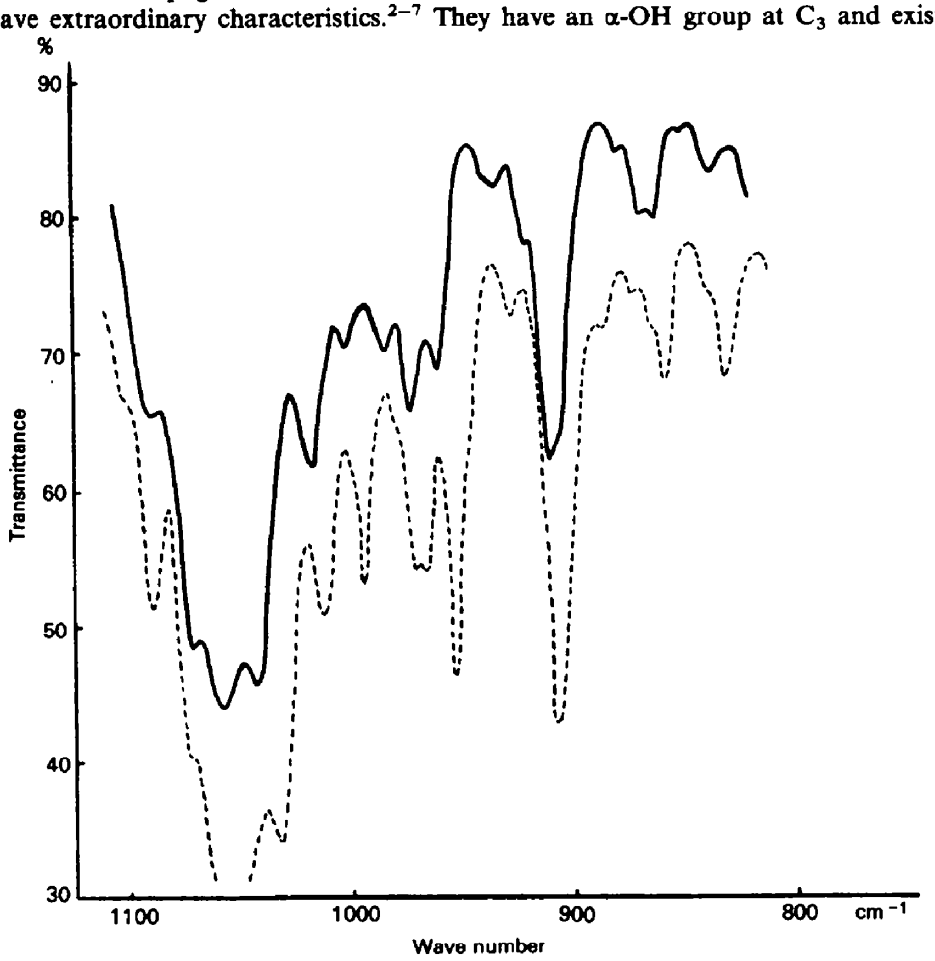


FIG. 1 The IR spectra of igagenin (solid line) and isonarthogenin (dotted line) in Nujol.

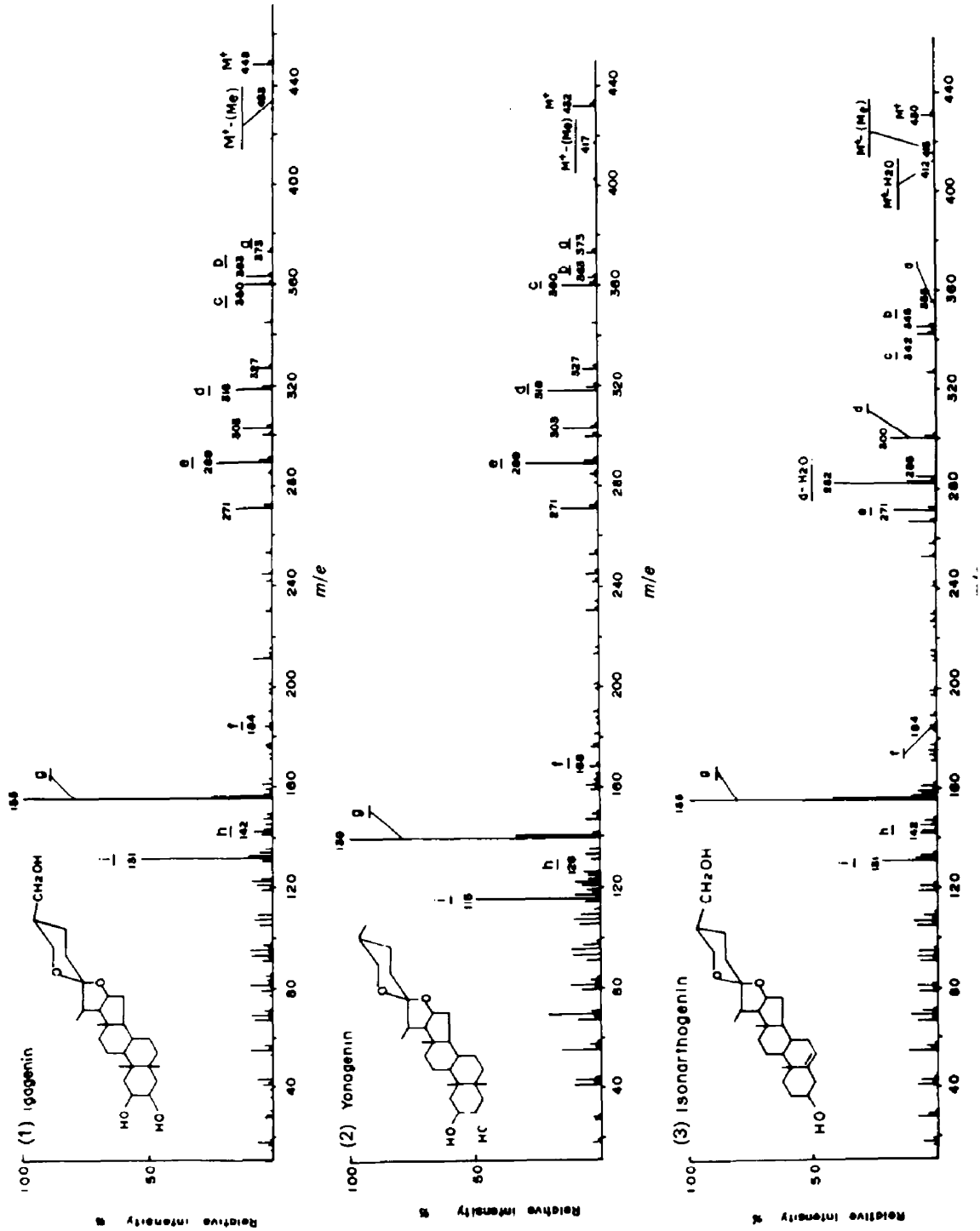
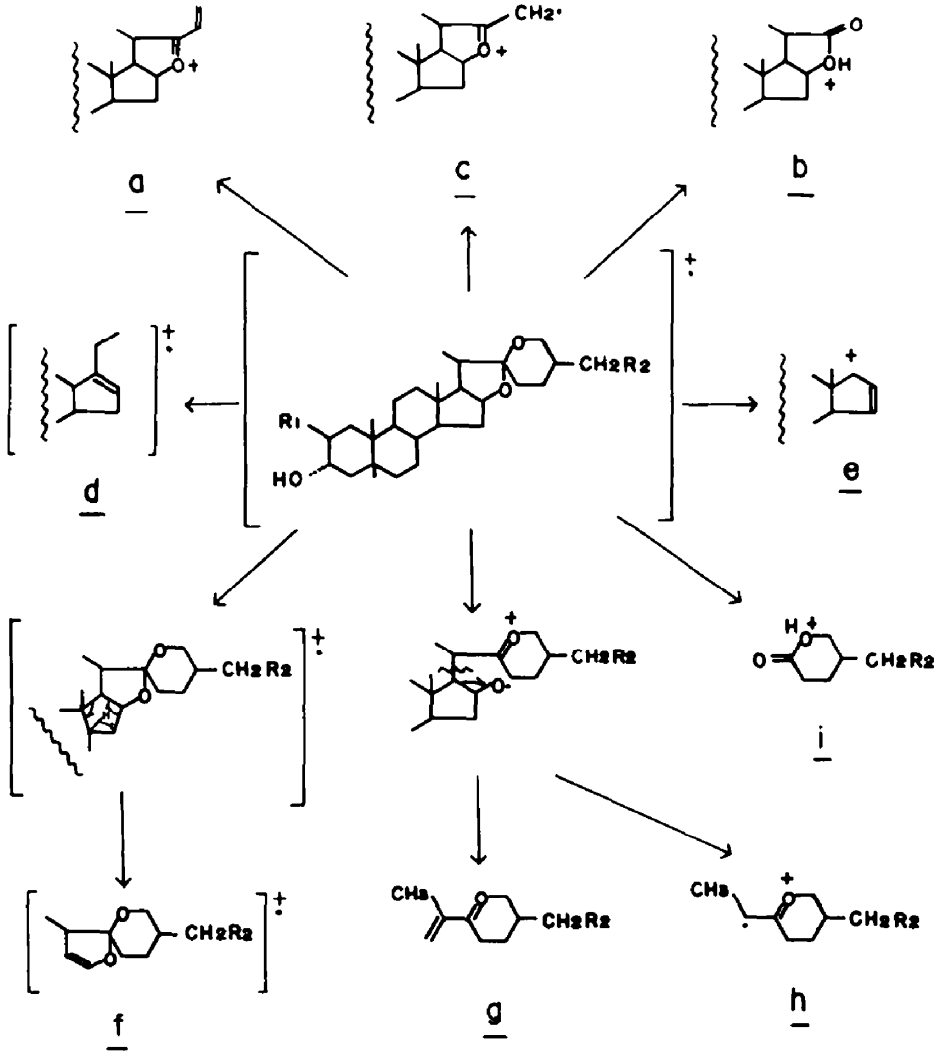


FIG. 2. The mass spectra of igagenin, yonogenin and isonarthogenin.

both as free sapogenins and aglycones of saponins.[1,8,9] The 3α-hydroxysapogenins have not been found in plants other than the two Japanese Dioscoreas, *D. tokoro* and *D. tenuipes* complex.[10–12] The newly isolated trihydroxysapogenin,[13] m.p. 253°,



R1 = R2 = OH : Igagenin
R1 = OH, R2 = H : Yonogenin
R1 = R2 = H, Δ^6 : Isonarthogenin

which was named igagenin according to the collection site* of the plant material, is therefore of interest regarding the presence of a 3α-hydroxyl group.

* The former Province of Iga, now Mie Pref.

This sapogenin is easily converted under mild conditions to a triacetate, m.p. 191–192°. The four bands characteristic of the steroidal sapogenins[14,15] do not appear in the IR spectrum of this sapogenin, but the two bands at 1017 and 911 cm^{-1} are very similar to those seen in isonarthogenin and isocarneagenin[16] and indicate an OH group at C_{27} (Fig. 1).

To elucidate the position of the hydroxyl groups, the mass and NMR spectra of this sapogenin were investigated. As shown in Fig. 2, the molecular ion at *m/e* 448 indicates a saturated trihydroxysapogenin. Peaks observed at *m/e* 373, 363, 360, 300

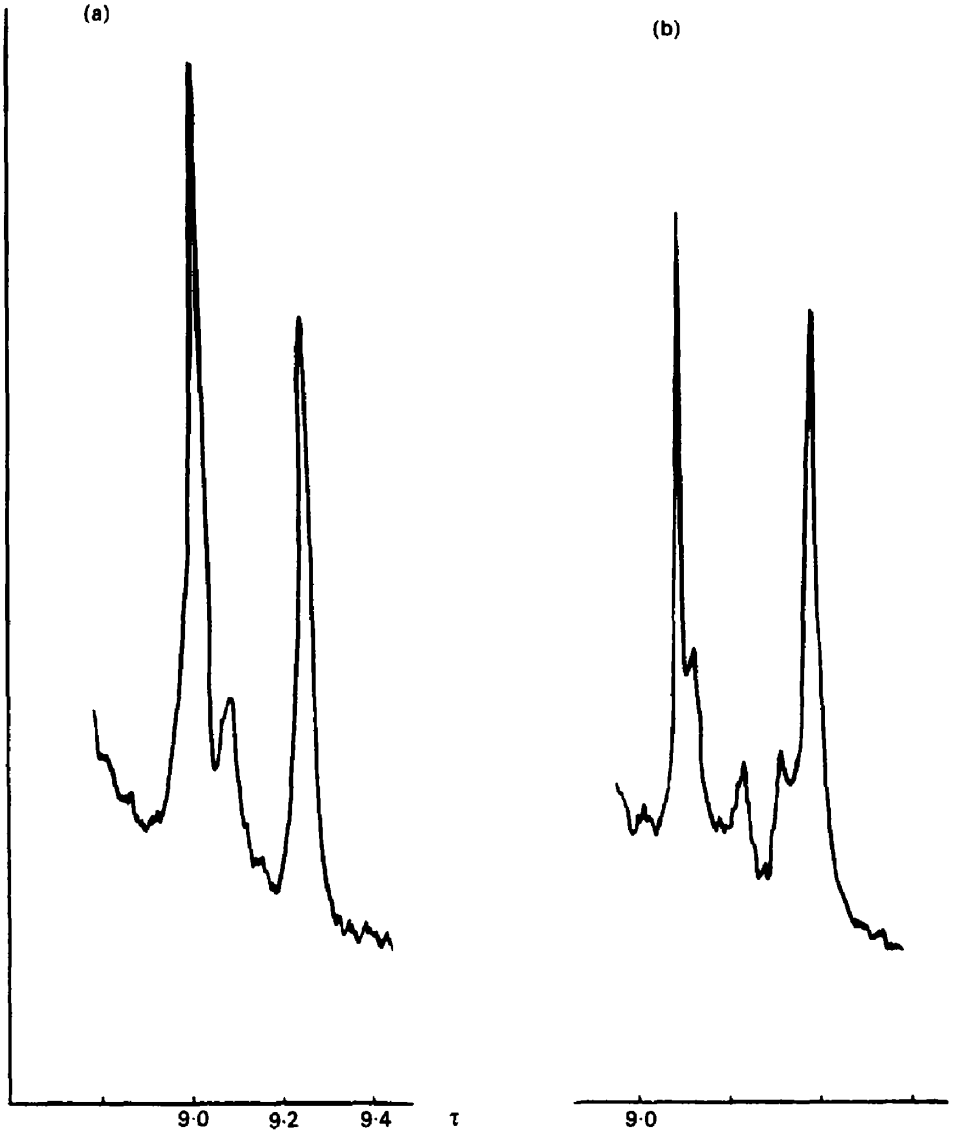
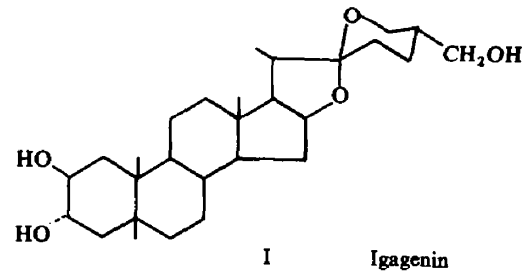


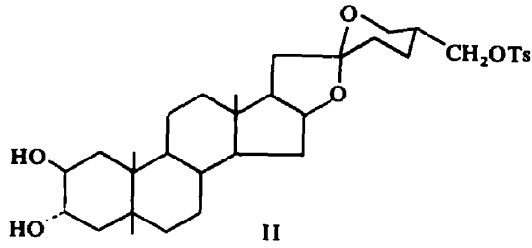
FIG. 3 The NMR spectra of (a) igagenin triacetate and (b) yonogenin diacetate in $CDCl_3$.

and 289 may correspond to the fragments which partially or completely lose the spiroketal side chain as reported by Bergström, *et al.*[17] and identical with those observed in the mass spectrum of yonogenin. The mass numbers of these peaks all shift as much as 2 mass units after the substitution of the H atoms of the OH groups



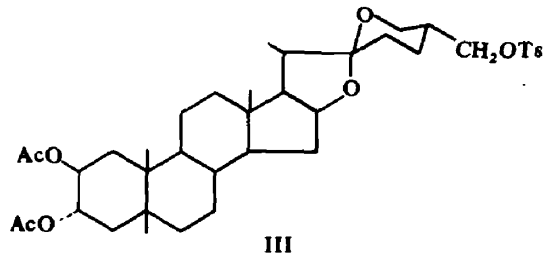
I Igagenin

TsCl



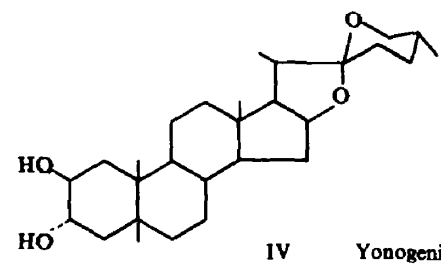
II

Ac_2O



III

LAH



IV Yonogenin

by deuterium. Furthermore, the peaks corresponding to the fragments of the side chain of the steroidal sapogenin[18] are not detected in the mass spectrum of this sapogenin and the peaks observed have mass numbers 16 mass units higher than those of the ordinary sapogenins. These peaks are identical with those of isonarthogenin (25D-spirost-5-ene-3β, 27-diol) and again suffer one mass unit shift after substitution with deuterium. Furthermore, a peak at 9·20–9·30 τ, which is observed in the NMR spectra of 25D-sapogenins, and considered to be one of the doublet signals specific to the C_{27}-Me group,[19] is not detected in that of igagenin. From these results, one of three OH groups of igagenin must be at C_{27}. In order to clarify the configuration of a C_{27}-hydroxymethyl group, igagenin was heated under reflux with hydrochloric acid in ethanol for 72 hr, but only the starting material was recovered and the epimerization product of the 27 hydroxylmethyl group was not obtained. This sapogenin was confirmed to be a derivative of the 25D-sapogenins.[20,21]

The position and configuration of the remaining two OH groups were decided as follows. Igagenin was converted to a monotosylate, which was then reduced with LAH. The mass and IR spectra of the acetate of this reduction product were identical with those of yonogenin diacetate. The structure of igagenin was confirmed to be 25D, 5β-spirostan-2β, 3α, 27-triol.

Igagenin is found only in the female flowers of *D. tokoro*. The concentration of this sapogenin in the other parts of this plant is considered to be very small even if it is present in all the parts. Although the physiological role of the sapogenins has not been elucidated, the marked concentration of this sapogenin in the female flowers suggests that this sapogenin may play some role in the reproduction of this plant.

EXPERIMENTAL

All m.ps are uncorrected. The IR, NMR and mass spectra were recorded with a Nippon Bunkô double-beam spectrophotometer model DS 201-B, Varian A-60 and Hitachi RMU-6E single focus mass spectrometer using a direct inlet system, respectively.

Deuterium exchange of active hydrogen for mass spectrometry. A slurry of the sample in D_2O was introduced into the direct inlet system of the mass spectrometer which was equilibrated with D_2O.

Treatment of igagenin with hydrochloric acid. Igagenin triacetate (8 mg) was dissolved in 30 ml 95% EtOH containing 7 ml 35% HCl and refluxed for 72 hr on an oil bath. The reaction mixture was then poured into water and extracted with $CHCl_3$. The reaction product (5 mg) was recrystallized from acetone to yield 3 mg of white needles, m.p. 238–243°. This was identified as igagenin by IR spectrum.

Igagenin monotosylate. Igagenin (17 mg) was dissolved in 2 ml pyridine containing 9 mg *p*-toluenesulfonyl chloride and allowed to stand overnight at room temp. The reaction mixture was poured into water and extracted with $CHCl_3$ to yield 21 mg of a white powder. This was subjected to preparative TLC on Kieselgel G plates and yielded 7 mg of a monotosylate and 9 mg of the starting material. The latter was again treated as above. The monotosylate thus obtained was very easily soluble in MeOH and formed a jelly in other solvents. This monotosylate (12 mg) was acetylated with Ac_2O in pyridine at room temp. This substance could not be crystallized as it is very soluble in MeOH and forms a jelly in other solvents. $[\alpha]_D^{22}$ −17·5° (*c*, 0·355, $CHCl_3$). (Found: C, 66·29; H, 7·93; S, 4·74. Calc. for $C_{38}H_{54}O_9S$: C, 66·44; H, 7·93; S, 4·67%); IR, cm^{-1} ($CHCl_3$): —OAc, 1737; —OTs, 1173, 1599.

The lithium aluminium hydride reduction of III. To 8 mg of III dissolved in 1 ml anhyd THF, 15 mg of LAH suspended in 1 ml anhyd THF was added dropwise with stirring, and the mixture was refluxed for 2 hr. Two drops AcOH were added and the reaction mixture poured into water and extracted with $CHCl_3$ to yield 7 mg of the reduction product. This was subjected to preparative TLC on a Kieselgel G plate to yield 2 mg of a diol fraction and 3 mg of a triol fraction. The IR spectrum of the former was identical with that of yonogenin. This substance was acetylated with pyridine and Ac_2O and recrystallized from MeOH to yield 1 mg white needles, m.p. 203–205°. This was identified as yonogenin diacetate by R_f value on a

thin-layer plate, IR and mass spectra. The triol fraction was recrystallized from MeOH to yield 2 mg white needles, m.p. 253–256°. This was identified as igagenin by IR spectrum.

Acknowledgement—The authors express their deep gratitude to Dr. H. Minato of this laboratory for his helpful advice.

REFERENCES

[1] Part LVI. A. Akahori, F. Yasuda, I. Okuno, M. Togami, T. Okanishi and T. Iwao, *Phytochem.* in press.
[2] K. Morita, *Pharm. Bull.* **5**, 494 (1957).
[3] K. Morita, *Bull. Chem. Soc. Japan* **32**, 791 (1959).
[4] K. Takeda, T. Okanishi and A. Shimaoka, *Chem. Pharm. Bull.* **6**, 532 (1958).
[5] T. Kubota, *Tetrahedron* **7**, 62 (1959).
[6] T. Kubota, *Chem. Pharm. Bull.* **7**, 898 (1959).
[7] A. Akahori, *Ann. Repts. Shionogi Res. Lab.* **10**, 153 (1960).
[8] A. Akahori, *Ibid.* **11**, 93 (1961).
[9] A. Akahori, *Ibid.* **11**, 97 (1961).
[10] A. Akahori, F. Yasuda and T. Okanishi, *Chem. Pharm. Bull.* **16**, 499 (1968).
[11] K. Takeda, T. Okanishi, A. Akahori and F. Yasuda, *Ibid.* **16**, 421 (1968).
[12] K. Takeda, G. Lukacz and F. Yasuda, *J. Chem. Soc.* in press.
[13] A. Akahori, I. Okuno, T. Okanishi and T. Iwao, *Chem. Pharm. Bull.*, in press.
[14] M. E. Wall, M. L. McClennan, C. R. Eddy and M. E. Klumpp, *Analyt. Chem.* **24**, 1337 (1952).
[15] R. N. Jones, *J. Am. Chem. Soc.* **75**, 158 (1953).
[16] K. Takeda, H. Minato, A. Shimaoka and Y. Matsui, *J. Chem. Soc.* **1963**, 4815.
[17] S. Bergström, R. Ryhage and E. Stenhagen, *Svensk Kemisk Tidskrift* **73**, 566 (1961).
[18] H. Budzikiewicz, J. M. Wilson and C. Djerassi, *Monatsh. Chem.* **93**, 1033 (1962).
[19] W. E. Rosen, J. B. Ziegler, A. C. Shabica and J. N. Shoolery, *J. Am. Chem. Soc.* **81**, 1687 (1959).
[20] R. E. Marker and E. Rohrmann, *Ibid.* **61**, 846 (1939).
[21] H. Minato and A. Shimaoka, *Chem. Pharm. Bull.* **11**, 867 (1963).