*Tetrahedron* Vol. 26, pp. 2365 to 2370 Pergamon Press 1970. Printed in Great Britain

# THE STRUCTURE AND ABSOLUTE CONFIGURATION OF SYRINGOPICROSIDE

## A NEW IRIDOID GLUCOSIDE FROM *SYRINGA VULGARIS* L.

Y. ASAKA, T. KAMIKAWA, T. TOKOROYAMA and T. KUBOTA

Faculty of Science, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka, Japan

(*Received in Japan* 17 *November* 1969; *Received in the UK for publication* 20 *January* 1970)

**Abstract**—The structure elucidation of syringopicroside, a new iridoid glucoside from *Syringa vulgaris* L., is described.

SOLVENT extraction of the dried leaves of *Syringa vulgaris* L., collected in Peking of People's Republic of China (or better results were obtained from the fresh leaves collected in Hokkaido of Japan), and chromatography led to the isolation of an amorphous bitter principle. Although all attempts to crystallize this compound failed, it appeared to be pure on TLC and was named syringopicroside **1**.* It gave a green coloration with a ferric chloride test and showed a positive Zimmerman test. On acetylation with acetic anhydride and pyridine, it gave a crystalline pentaacetate **2**, $C_{34}H_{40}O_{16}$. The IR spectrum of **2** showed bands attributable to CO groups at 1740 and 1700 $cm^{-1}$, a conjugated double bond at 1640 $cm^{-1}$ and aromatic absorption 1510 and 845 $cm^{-1}$. The UV spectrum showed maxima at 221 ($\varepsilon$ 11,500) and 271 m$\mu$ ($\varepsilon$ 420). The UV spectrum ($\lambda_{max}$ 221 m$\mu$) and the NMR spectrum ($\delta^{CDCl_3}$ 7·42 ppm, br.s, —OCOC=CH--O—) suggest the presence of iridoid structure.[2-10] Kuhn-Roth oxidation of **2** indicated the presence of one C—Me group in addition to that of five Ac groups. In accord with this, the NMR spectrum showed signals due to five Ac groups (1·84, 2·02, 2·05, 2·12 and 2·37 ppm) and a secondary Me group (1·18 ppm, d, $J$ = 6 Hz).

Mild hydrolysis of **2** with hydrochloric acid gave an amorphous powder, identical with syringopicroside **1** on TLC, which showed a CO band at 1730 $cm^{-1}$ and bands characteristic to the iridoid compounds at 1685 and 1630 $cm^{-1}$ in the IR spectrum.[3, 5] On acetylation the pentaacetate **2** was recovered. Hydrolysis of the pentaacetate **2** with *p*-toluenesulfonic acid in ethanol gave a crystalline tetraacetate **3**, $C_{32}H_{38}O_{15}$, which showed a positive ferric chloride test. The UV spectrum showed absorption maxima at 227·5, 280 and 286 (infl) m$\mu$; $\varepsilon$ 15,700, 2200 and 1900 resp. in ethanol, on addition of sodium hydroxide a large shift in the absorption spectrum was observed ($\lambda_{max}$ 241 and 290 m$\mu$; $\varepsilon$ 31,100 and 9900 resp). These results suggest the presence of one phenol acetate in addition to four Ac groups in **2**.

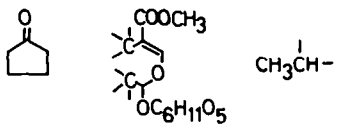
* In 1862, Kromayer[1] obtained a bitter substance, named syringopicrin, from the leaves of *S. vulgaris* L. As the identity of his compound, though it was not obtained in pure state, with ours is quite easily supposed, we want to use the name syringopicroside for our bitter principle.

The presence of a ketone group in **2** was indicated by a negative Cotton effect in the ORD measurement and the formation of monosemicarbazone ($\lambda_{max}$ 230 mμ; ε 2330). From its UV spectrum, it is suggested that this ketone group is not conjugated. The pentaacetate **2** was reduced by sodium borohydride to give an amorphous alcohol, which on acetylation gave a crystalline hexaacetate **4**.

The β-glucosidic nature of **1** was confirmed by the production of D-glucose on enzymatic hydrolysis with emulsin. Acid hydrolysis of the pentaacetate **2** merely resulted in the removal of the Ac groups, while the hydrolysis with sodium methoxide or barium hydroxide gave a mixture of acids and an alcohol **5**, $C_8H_{10}O_2$.

The alcohol **5** showed green coloration towards ferric chloride, suggesting the presence of a phenolic group. The UV spectrum of **5** ($\lambda_{max}$ 224·5, 279, 286 (sh) mμ; ε 6300, 3000, 2000 resp) is similar to that of *p*-cresol ($\lambda_{max}$ 279 and 286 mμ; ε 1950 and 1780 resp). The NMR spectrum of **5** showed the presence of an $A_2X_2$ system due to protons of $ArCH_2CH_2OH$ group (δ 3·07, 4·66 ppm, each t, $J$ = 8·2 Hz) and an $A_2B_2$ system due to protons on *p*-disubstituted benzene ring.* These data are consistent with those of *p*-hydroxy phenethylalcohol. The identity was confirmed by comparison with the authentic sample.[11]

Esterification of the mixture of acids with diazomethane followed by chromatography afforded a methyl ester A **6**, $C_{17}H_{24}O_{10}$ and a methyl ester B **7**, $C_{18}H_{28}O_{11}$. The spectral data of **6** suggest the presence of cyclopentanone ring [$\nu_{max}$ 1750 $cm^{-1}$; $\lambda_{max}$ 290 mμ (ε 30)], an enol–ether group conjugated with carbomethoxy function ($\nu_{max}$ 1680, 1645 $cm^{-1}$; $\lambda_{max}$ 235 mμ (ε 10,500); $\delta^{D_2O}_{DSS}$ 3·77 (3H, s), 5·75 (1H, d, $J$ = 3 Hz, —O—CH—O—Glu), and 7·64 ppm (1H, d, $J$ = 1·5 Hz, —O—COC=CH—O—) and a secondary methyl group ($\delta^{D_2O}_{DSS}$ 1·13 ppm, d, $J$ = 6 Hz). All of the oxygen atoms of **6** are thus accounted for by the following partial structure:




and hence **6** must be bicyclic.

Thus the keto ester A has one of the structures **6** or **8** (verbenalin).[5] The secondary methyl group is placed at C-8 from biogenetic reasons.[12, 13] Of these formulations, **8** was excluded by direct comparison of the keto ester with verbenalin.[5, 14] Thus the keto ester **6** should have the structure of 7-keto loganin.

In accordance with this conclusion, the acetylation of **6** gave a tetraacetate **9** (R = Ac), whose physical properties were identical with those of tetraacetyl 7-keto loganin,[3, 15, 16] which was obtained by oxidation of loganin with Jones' reagent followed by acetylation.

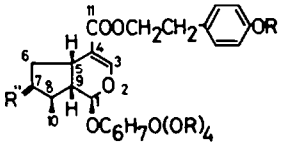
The methyl ester B **7** had no ultraviolet absorption band at about 235 mμ and lacked an infrared peak near 1640 $cm^{-1}$. The NMR spectrum of **7** showed no signal due to a grouping —OCOC=CH—O, but a new three protons singlet at 3·55 ppm ($OCH_3$),

* The NMR spectrum was run in $CF_3COOH$ with TMS as an internal standard.

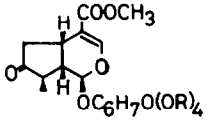
one proton doublet at 2·60 ppm ($J$ = 9 Hz, $-\overset{|}{C}H-\overset{|}{C}H-COO-$) and one proton doublet at 5·30 ppm ($J$ = 9 Hz, $-O-\underset{|}{\overset{OCH_3}{\overset{|}{C}}}H-\underset{|}{C}H-$). These data suggest the addition of methanol to the conjugated double bond of the dihydropyran ring of **6**. Axial attack of a methoxide ion from α-side of the molecule would give an ion **10**, which then gave **11** having di-axial bulky groups. Inversion of the tetrahydropyran ring would give the more stable conformer **12**, as the coupling constant of the C-3-proton signal indicates ($J$ = 9 Hz).[17] The inversion would also change the conformation of the glucose residue at C-1 from quasi-equatorial to quasi-axial, indicated by the observed singlet C-1 proton signal ($\delta$ 5·60 ppm, the dihedral angle between the C-1 and C-9 protons to be approximately 90°).[17] Hence the methyl ester B is assigned the structure **7**.

Since syringopicroside has a free phenolic group, the ester linkage of **1** is made through the bonding between the alcoholic OH group of **5** and the carboxyl group of **6**. Therefore, the structure of syringopicroside including absolute configuration is best represented by **1**. The possibility that syringopicroside would have less stable configuration at C-8 and this less stable form, on hydrolysis with sodium methoxide, would have been epimerized to the stable form (i.e. **6**) is excluded from the fact that **2** and **9** showed similar Cotton effects on their ORD curves.

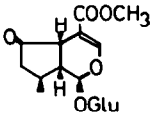
Syringopicroside is the first example of 7-keto loganin derivative in nature.



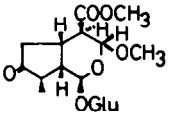
1 : R=R'=H, R''=O
2 : R=R'=Ac, R''=O
3 : R=Ac, R'=H, R''=O
4 : R=R'=Ac, R''=



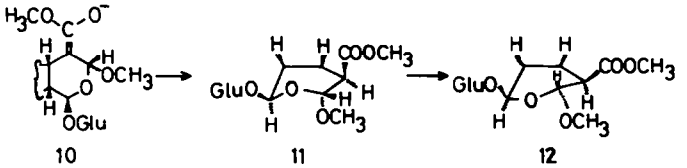
6 : R=H
9 : R=Ac



8



7



10 11 12

## EXPERIMENTAL

M.ps were uncorrected. The IR spectra were measured as Nujol mulls on a Nippon Bunko IR-S spectrometer and the UV spectra were determined in ethanol solutions on a Hitachi EPR-S recording spectrometer. The NMR spectra were taken in $CDCl_3$ solns on a JNMC-60 spectrometer. Chemical shifts were reported in $\delta$-value using TMS as an internal references. Mallinckrodt silicic acid was used for column chromatography. Microanalyses were carried out by Mr. J. Goda of Microanalytical Laboratory, Faculty of Science, Osaka City University.

*Isolation of syringopicroside* **1**. The dried leaves of *S. vulgaris* L. (33·0 Kg) were extracted with boiling water. The combined extracts were evaporated at 50–55° under reduced press to about 1/10 of the original volume, and the resulting soln was extracted with ethyl acetate by use of a vibromixer. The extract was dried ($Na_2SO_4$), and the solvent was removed under reduced press, leaving crude material (560 g). A part of crude material (65 g) was chromatographed on neutral alumina (Activity 3, 2 Kg). Elution with 80% dil acetone gave a bitter substance as an amorphous powder (25·5 g).

*Pentaacetyl syringopicroside* **2**. The bitter substance (54·2 g) was acetylated with $Ac_2O$ (250 ml) and pyridine (160 ml). The crude product was chromatographed on silicic acid (2 Kg). Elution with $CHCl_3$ gave **2** as pentagonal plates (11·8 g), m.p. 156–156·5° (from EtOH), $[\alpha]_D^{20\cdot6}$ −116·5° ($c$ = 1·0, $CHCl_3$), $\lambda_{max}$ 221, 271 mμ ($\varepsilon$ 11,500, 420 resp), $\nu_{max}$ 1740, 1700, 1640, 1510 and 845 $cm^{-1}$, $\delta^{CDCl_3}$ 1·18 (3H, d, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 1·84, 2·02, 2·05, 2·12 and 2·37 (each 3H, s, $CH_3COO-$), 2·98, 4·38 (each 2H, t, $J$ = 6·8 Hz, $-CH_2CH_2-$), 5·51 (1H, s, $-O-\underset{|}{C}H-O-$), 7·10 (4H, arom. protons), 7·42 ppm (1H, s, $-OCO\underset{|}{C}=CH-O-$). Kuhn-Roth oxidation: C-Me, 12·33, $C_{28}H_{22}O_{16}(CH_3)_6$ requires: C-Me, 12·63%. (Found: C, 58·03; H, 5·75, $C_{34}H_{40}O_{16}$ requires: C, 57·94; H, 5·72%). ORD (MeOH): $[\phi]_{350}$ −5200, $[\phi]_{320}$ −10,000, $[\phi]_{308}$ −11,200, $[\phi]_{279}$ −2933, $[\phi]_{215}$ +15,467, $[\phi]_{205}$ +7467.

*Treatment of syringopicroside* **1** *with emulsin*. To a soln of emulsin (504 mg) in acetate buffer soln (pH 5·3, 60 ml) and water (40 ml), syringopicroside (1·486 g) was added. After 24 hr, the soln was extracted with ether overnight. The extract was washed with water, dried ($MgSO_4$) and the solvent was removed under reduced press, leaving crude aglycone (718 mg). The aqueous soln was evaporated to dryness under reduced press and the residue was dissolved in a small amount of water. The presence of glucose in the residue was confirmed by PPC. Glucosazone was prepared by the usual method, m.p. 195° (dec), undepressed on admixture with the authentic sample.

*Monosemicarbazone of* **2**. Pentaacetyl syringopicroside (198 mg) was dissolved in EtOH (6 ml). Water was added until the soln was faintly turbid, and the turbidity was removed with a few drops of EtOH. The semicarbazide hydrochloride (33 mg) and sodium acetate (33 mg) were added and after allowing to stand for 24 hr, the crystalline ppt was collected and recrystallized from EtOH to give a monosemicarbazone (172 mg), m.p. 184–185°. (Found: C, 55·07; H, 5·74; N, 5·57. $C_{35}H_{43}O_{16}N_3$ requires: C, 55·18; H, 5·69; N, 5·52%).

*Treatment of* **2** *with acid* (1). Pentaacetyl syringopicroside (200 mg) was dissolved in MeOH (10 ml) and 1N-HCl (10 ml) was added. The soln was heated under reflux for 1 hr. The soln was neutralized and extracted with EtOAc. The combined extracts were washed with water, dried and the solvent was removed under reduced press, leaving an amorphous material (137 mg). $[\alpha]_D^{14\cdot8}$ −115° ($c$ = 1·0, $H_2O$), $\nu_{max}$ 3400, 1730, 1685, 1630 and 850 $cm^{-1}$, $\delta^{D_2O}$ 1·14 (3H, d, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 2·72, 4·15 (each 2H, t, $J$ = 6·2 Hz, $-CH_2CH_2-$), 5·48 (1H, d, $J$ = 1·5 Hz, $-O-\underset{|}{C}H-O-$), 6·82 (4H, q, $J$ = 8·2 Hz, arom. protons), 7·35 (1H, s, $-OCO\underset{|}{C}=CH-O-$).

The starting material was recovered on acetylation of the amorphous substance, m.p. 155–156° (from EtOH).

*Treatment of* **2** *with acid* (2). Pentaacetyl syringopicroside (1 g) was dissolved in ethanol (10 ml) and *p*-toluenesulfonic acid (100 mg) was added. The soln was heated under reflux for 15 min. The soln was diluted with water and extracted with $CHCl_3$. The extract was washed with $NaHCO_3$aq and water, dried and the solvent was removed under reduced press, leaving an amorphous material. The crude material was chromatographed on silicic acid (20 g). Elution with $CHCl_3$-$Me_2CO$ (9:1) gave **3** (230 mg), m.p. 172–174° (from EtOH), $\lambda_{max}$ 227·5, 280 and 286 (infl) mμ ($\varepsilon$ 15,700, 2200 and 1900 resp), $\lambda_{max}^{OH-EtOH}$ 241, 290 mμ ($\varepsilon$ 31,100, 9900 resp). (Found: C, 58·05; H, 5·86. $C_{32}H_{38}O_{15}$ requires: C, 58·00; H, 5·78%).

The starting material was recovered on acetylation of **3**.

*Treatment of* **2** *with base* (1). Pentaacetyl syringopicroside (138 mg) was dissolved in MeOH (8 ml) and

0·1N NaOH (13 ml) was added. The soln was heated under reflux for 30 min. The soln was neutralized and extracted with EtOAc. The combined extracts were washed with water, dried and the solvent was removed under reduced press, leaving an amorphous material (64 mg). The starting material was recovered on acetylation of the amorphous substance.

*Treatment of* **2** *with base* (2). Pentaacetyl syringopicroside (3 g) and NaOMe (1·15 g) were dissolved in abs MeOH (30 ml). The soln was refluxed for 3 hr. The cooled soln was passed through an ion-exchange resin column (Amberlite IRC-50). The solvent was removed under reduced press, leaving an amorphous substance (2·39 g). The residue was dissolved in water and extracted continuously with ether overnight. The extract was washed with water, dried and the solvent was removed under reduced press, leaving crude crystalline material. Recrystallization from benzene yielded *p*-hydroxyphenethylalcohol (480 mg), m.p. 92–93° (lit. m.p. 91–92°),[11] $\lambda_{max}$ 224·5, 279 and 286 mμ (ε 6300, 3000 and 2000 resp), $\nu_{max}$ 3400, 3150, 1600, 1500 and 820 $cm^{-1}$, $\delta^{CF_3COOH}$ 3·07, 4·65 (each 2H, t, $J$ = 6·8 Hz, $-CH_2CH_2-$), and 7·09 ppm (4H, q, $J$ = 8·2 Hz, arom protons). The IR spectrum was identical with that of the authentic sample.

The aqueous soln was evaporated to dryness under reduced press and the residue was methylated with ethereal diazomethane to give an amorphous solid (1·67 g). The solid was chromatographed on charcoal–celite (58 g: 58 g). Elution with 35% dil EtOH gave a hydrogen methoxide **7**, (606 mg), m.p. 187·5–188° (from EtOH), $\nu_{max}$ 3500, 3300 and 1740 $cm^{-1}$, $\delta^{D_2O}_{DSS}$ 1·11 (3H, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 3·55 (3H, s, $-OCH_3$), 3·79 (3H, s, $-COOCH_3$) and 5·60 ppm (1H, s, $-O-\underset{|}{C}H-O-$). (Found: C, 51·29; H, 6·77. $C_{18}H_{28}O_{11}$ requires: C, 51·42; H, 6·71%).

Further elution with 40% dil EtOH gave 7-keto loganin **6**, (247 mg), m.p. 192–193° (lit. m.p. 193–195°),[15] $[\alpha]_D^{17}$ −113° ($c$ = 1·0, $H_2O$), $\lambda_{max}$ 235, 290 mμ (ε 10,500, 30 resp), $\nu_{max}$ 3300, 1750, 1680 and 1645 $cm^{-1}$, $\delta^{D_2O}$ 1·13 (3H, d, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 3·77 (3H, s, $COOCH_3$), 5·75 (1H, d, $J$ = 3 Hz, $-O\underset{|}{C}H-O-$), and 7·64 ppm (1H, d, $J$ = 1·5 Hz, $-OCO\underset{|}{C}=CH-O-$). (Found: C, 52·39; H, 6·32. $C_{17}H_{24}O_{10}$ requires: C, 52·57; H, 6·23%).

*Treatment of* **2** *with base* (3). Pentaacetyl syringopicroside (2·112 g) was heated with $Ba(OH)_2$ $8H_2O$ (9·5 g) soln under reflux for 4 hr. The soln was acidified with 6N $H_2SO_4$ and the ppt was removed by centrifugation. The aqueous soln was extracted continuously with ether overnight. The extract was washed with water, dried and the solvent was removed under reduced press to give a crystalline material. Recrystallization from benzene yielded *p*-hydroxyphenethylalcohol **5** (323 mg), m.p. 92–93°. The IR spectrum was identical with that of the authentic sample. The aqueous solution was evaporated to dryness under reduced press and the residue was treated with ethereal diazomethane. The solvent was removed under reduced press, leaving an amorphous residue (1·028 g). The residue was chromatographed on charcoal–celite (36 g: 36 g). Elution with 40% dil EtOH gave **6**, (141 mg), m.p. 192–193° (from EtOH).

*Tetraacetyl 7-keto loganin* **9**. 7-Keto loganin **6** (182 mg) was acetylated by the usual manner to give **9** (126 mg), m.p. 143° (from EtOH), (lit. 117°,[15] 104–106°[16]), undepressed on admixture with the authentic sample. $[\alpha]_D^{30}$ −130° ($c$ = 1·0, $CHCl_3$), $\nu_{max}$ 1752, 1735, 1700 and 1630 $cm^{-1}$, $\delta^{CDCl_3}$ 1·15 (3H, d, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 1·87, 1·98, 2·01 and 2·08 (each 3H, s, $-OCOCH_3$), 3·70 (3H, s, $-COOCH_3$), 5·43 (1H, d, $J$ = 3 Hz, $-O-\underset{|}{C}H-O-$), and 7·38 ppm (1H, d, $J$ = 1·5 Hz, $-OCO\underset{|}{C}=CH-O-$). (Found: C, 53·79; H, 6·19. $C_{25}H_{32}O_{14}$ requires: C, 53·95; H, 5·80%).

The IR and NMR spectra and the ORD curve were identical with those of the authentic sample.

*Sodium borohydride reduction of* **2**. Pentaacetyl **2** (2·758 g) was dissolved in MeOH (50 ml) and $NaBH_4$ (204 mg) was added. After allowing to stand for 1 hr at room temp, the solvent was evaporated under reduced press and the residue was extracted with EtOAc. The solvent was evaporated to give an amorphous material. The amorphous material was acetylated by the usual manner to give **4** (2·729 g), m.p. 118° (from isopropanol), $\nu_{max}$ 1740, 1700, 1635 and 1500 $cm^{-1}$, $\delta^{CDCl_3}$ 1·11 (3H, d, $J$ = 6 Hz, $-\underset{|}{C}HCH_3$), 1·87, 1·97, 2·00, 2·03, 2·09, and 2·29 (each 3H, s, $-OCOCH_3$), 2·95, 4·55 (each 2H, t, $J$ = 7·5 Hz, $CH_2CH_2-$), 5·28 (1H, d, $J$ = 3 Hz, $-O-\underset{|}{C}H-O-$), 7·20 (4H, m, arom. protons) and 7·33 ppm (1H, d, $J$ = 1·5 Hz, $-OCO\underset{|}{C}=CH-O-$). (Found: C, 57·90; H, 6·14. $C_{36}H_{44}O_{17}$ requires: C, 57·75; H, 5·92%).

*Acknowledgements*—We thank Prof. T. Irie of Hokkaido University and Dr. Kuo Mo-jo, President of

Academia Sinica, People's Republic of China for collecting the leaves of *Syringa vulgaris* L. We also thank Prof. H. Inouye for spectral data and sample of 7-keto loganin and Dr. K. Kuriyama for rotatory dispersion measurement. We are grateful to the Ministry of Education and Takeda Chemical Industries, Ltd. for support of this research.

## REFERENCES

[1] A. Kromayer, *Arch. Pharm.* **159**, 18 (1862)
[2] O. Halpern and H. Schmid, *Helv. Chim. Acta* **41**, 1109 (1958)
[3] K. Sheth, E. Ramstad and J. Wolinsky, *Tetrahedron Letters* 394 (1961)
[4] C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.* **26**, 1192 (1961)
[5] G. Büchi and R. E. Manning, *Tetrahedron* **18**, 1049 (1962)
[6] H. Inouye, T. Arai and Y. Miyoshi, *Chem. Pharm. Bull.* **12**, 888 (1964)
[7] L. H. Briggs, B. F. Cain, P. W. LeQuensne and J. N. Shoolery, *J. Chem. Soc.* 2595 (1965)
[8] H. Inouye, S. Ueda, M. Hirabayashi and N. Shimokawa, *Yakugaku Zasshi* **86**, 943 (1966)
[9] O. Sticher and H. Schmid, *Helv. Chim. Acta* **52**, 478 (1969)
[10] H. Inouye, S. Saito, H. Taguchi and T. Endo, *Tetrahedron Letters* 2347 (1969)
[11] S. Yamada, T. Fujii, K. Takagi, Y. Gomi and S. Matsushita, *Chem. Pharm. Bull. Tokyo* **11**, 258 (1963)
[12] A. G. Horodysky, G. R. Waller and E. J. Eisenbraun, *J. Biol. Chem.* **244**, 3110 (1969)
[13] E. Leete, *Accounts of Chemical Research* **2**, 59 (1969)
[14] A. R. Battersby, R. S. Kapil and R. Sauthgate, *Chem. Commun.* 131 (1968)
[15] H. Inouye, T. Yoshida and S. Tobita, *Tetrahedron Letters* 2945 (1968)
[16] S. Brechbuehler, C. J. Coscia, P. Loew, Ch. von Szczepanshi and D. Arigoni, *Chem. Commun.* 136 (1968)
[17] J. W. Emsley, J. Feeney and L. H. Sutcliffe, *High Resolution Nuclear Magnetic Resonance Spectroscopy* Vol. 2; p. 703. Pergamon Press, London (1965)