[Chem. Pharm. Bull. 25(5)1140—1143(1977)]

UDC 547.455.04 : 543.422.25

Thiosugars. IV.[1] 2,3-Dideoxy-2,3-epithio-D-allose and 2,3-Dideoxy-2,3-epithio-D-mannose

TADATOSHI YAMAGUCHI and MASAHARU KOJIMA

Faculty of Pharmaceutical Sciences, Kyushu University[2]

(Received August 17, 1976)

2,3-Dideoxy-2,3-epithio-β-D-allofuranose (**3c**) and 2,3-dideoxy-2,3-epithio-α-D-mannofuranose (**4c**) were obtained, respectively, by the hydrolyses of methyl 2,3-dideoxy-2,3-epithio-α-D-allopyranoside (**1a**) and methyl 2,3-dideoxy-2,3-epithio-α-D-mannopyranoside (**2a**) with Amberlite CG-120(H^+) in H_2O. The structures of these reducing sugars were confirmed by nuclear magnetic resonance spectroscopy.

Keywords——2,3-dideoxy-2,3-epithio-β-D-allose; 2,3-dideoxy-2,3-epithio-α-D-mannose; Amberlite CG-120; hydrolysis of methyl 2,3-dideoxy-2,3-epithio-allopyranoside; hydrolysis of methyl 2,3-dideoxy-2,3-epithio-mannopyranoside

In the previous paper,[3] we reported the syntheses of methyl 2,3-dideoxy-2,3-epithio-α-D-allo(and α-D-manno)pyranoside (**1a** and **2a**) and methyl 2,3-dideoxy-2,3-epithio-β-D-allo(and α-D-manno)furanoside (**3a** and **4a**). However, any reducing sugar having epithio group in it has not been synthesized so far. This paper describes the hydrolyses of **1a**, **2a**, **3a** and **4a** using cation exchange resin as a mild acid catalyst to obtain the reducing 2,3-epithio hexoses.

Most of epithio compounds should be treated with care to avoid violent conditions such as usual hydrolysis of methyl glycosides with strong acid because of the sensitivity of epithio group to acid. It was previously found by us that 2,3-epithio-pyranosides (**1a** and **2a**) isomerize into the corresponding furanosides (**3a** and **4a**), respectively, by heating at 55° in 80% MeOH in the presence of cation exchange resin,[3] and that the transformation proceeded *via* the formation of carbonium ion at C-1 as a result of the protonation on the C_1-methoxy group by the inductive effect of the S-substituent at C-2.[1] This finding could be applied to the hydrolysis of **1a** or **2a**. Namely, treatment of **1a** or **2a** with cation exchange resin using H_2O instead of 80% MeOH as solvent should induce the formation of each reducing sugars. Fortunately, such a proposal was satisfactorily applicable to the hydrolyses of **1a** and **2a**.

Hydrolysis of **1a** with Amberlite CG-120(H^+) in H_2O at 55° gave crystalline 2,3-dideoxy-2,3-epithio-β-D-allofuranose (**3c**) (mp 98—99°) in a yield of 76.6%. Acetylation of **3c** gave 1,5,6-tri-O-acetyl-2,3-dideoxy-2,3-epithio-β-D-allofuranose (**3d**), and on treatment of **3c** with Amberlite CG-120(H^+) in MeOH, **3a** was obtained.

The structure (**3c**) was readily identified from its proton magnetic resonance (PMR) spectrum. The signal (doublet, J=5.2 Hz) ascribed to the proton of C_1-hydroxyl group appeared at δ 6.69. Since the C_1-H (doublet, J=5.2 Hz) at δ 5.31 does not couple with C_2-H (doublet, $J_{2,3}$=5 Hz) at δ 3.41, the configuration of C_1-H to C_2-H is determined to be *trans*. Accordingly, the configuration of C_1-hydroxyl group can be assigned as β. As the signal of C_3-H ($J_{2,3}$=5 Hz) at δ 3.79 coupled with C_2-H is doublet, the $J_{3,4}$ value of C_3-H is estimated as zero.

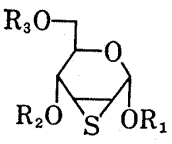
1) Part III: T. Yamaguchi and M. Kojima, *Carbohyd. Res.*, in press.
2) Location: *Maidashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan.*
3) M. Kojima, M. Watanabe, T. Yamaguchi, S. Ishimaru, and T. Taguchi, *Yakugaku Zasshi*, **96**, 1241 (1976).

On the other hand, the $J_{3,4}$ values of C_3-H in **3a** and its 5,6-di-O-benzoate (**3b**) are also zero,[4] respectively. Consequently, it is reasonable to assume that the structure of **3c** is made up of a furanoid ring.

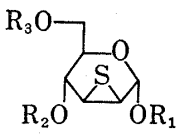
In a similar treatment of **2a** with Amberlite CG-120(H^+) in H_2O, syrupy 2,3-dideoxy-2,3-epithio-α-D-mannofuranose (**4c**) was obtained in a yield of 65.5%. Benzoylation of **4c** gave syrupy 1,5,6-tri-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-mannofuranose (**4d**), and treatment of **4c** with Amberlite CG-120(H^+) in MeOH afforded **4a**.

The PMR spectrum of **4c** showed the signal (doublet, J=6 Hz) of the proton of the C_1-hydroxyl group at δ 6.63. The $J_{1,2}$ value (zero) of C_1-H (doublet, J=6 Hz) at δ 5.24 indicates the *trans*-configuration of C_1-H to C_2-H. Thus the C_1-hydroxyl group of **4c** is in an α-configuration. From the doublet signal ($J_{2,3}$=5 Hz) of C_2-H at δ 3.39 and the quartet signal ($J_{2,3}$=5 Hz, $J_{3,4}$=3 Hz) of C_3-H at δ 3.74, the $J_{3,4}$ value of C_3-H is estimated to be 3 Hz. As the $J_{3,4}$ values of C_3-H in **4a** and its 5,6-di-O-benzoate (**4b**) are 3 Hz,[5] respectively, the structure of **4c** was also confirmed to have a furanoid ring.

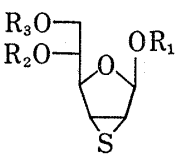
Treatment of **3a** and **4a** with Amberlite CG-120(H^+) in H_2O also produced **3c** and **4c**, respectively. Therefore, it has become apparent that the +I effect of the S-substituent at C-2 is also a cause for the transformation of **1a**, **2a**, **3a** and **4a** into reducing sugars with a furanoid ring (**3c** and **4c**) on hydrolysis with acid.



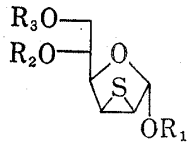
1a : R_1=CH_3, R_2=R_3=H
1b : R_1=CH_3, R_2=R_3=COC_6H_5



2a : R_1=CH_3, R_2=R_3=H
2b : R_1=CH_3, R_2=R_3=COC_6H_5



3a : R_1=CH_3, R_2=R_3=H
3b : R_1=CH_3, R_2=R_3=COC_6H_5
3c : R_1=R_2=R_3=H
3d : R_1=R_2=R_3=$COCH_3$



4a : R_1=CH_3, R_2=R_3=H
4b : R_1=CH_3, R_2=R_3=COC_6H_5
4c : R_1=R_2=R_3=H
4d : R_1=R_2=R_3=COC_6H_5

Experimental[6]

Compounds (**1a**, **2a**, **3a** and **4a**) were prepared as described in the previous paper.[3] Only PMR data are described below.

Methyl 2,3-Dideoxy-2,3-epithio-α-D-allopyranoside (**1a**)——PMR δ: 2.54 (2H, deuterium exchangeable, OH), 3.40 (3H, singlet, OCH_3), 4.21 (1H, quartet, J=4 Hz, J=9 Hz, C_5–H), 5.13 (1H, doublet, $J_{1,2}$=5 Hz, C_1–H).

Methyl 2,3-Dideoxy-2,3-epithio-α-D-mannopyranoside (**2a**)——PMR δ: 2.24 (2H, deuterium exchangeable, OH), 3.09 (1H, doublet, $J_{2,3}$=6 Hz, C_2–H), 3.21 (1H, doublet, $J_{2,3}$=6 Hz, C_3–H), 3.49 (3H, singlet, OCH_3), 4.16 (1H, doublet, J=8 Hz, C_5–H), 5.06 (1H, singlet, C_1–H).

Methyl 2,3-Dideoxy-2,3-epithio-β-D-allofuranoside (**3a**)——PMR δ: 2.86 (2H, deuterium exchangeable, OH), 3.44 (1H, doublet, $J_{2,3}$=4.5 Hz, C_2–H), 3.50 (3H, singlet, OCH_3), 3.77 (1H, doublet, $J_{2,3}$=4.5 Hz, C_3–H), 4.32 (1H, doublet, $J_{4,5}$=4 Hz, C_4–H), 5.13 (1H, singlet, C_1–H).

Methyl 2,3-Dideoxy-2,3-epithio-α-D-mannofuranoside (**4a**)——PMR δ: 2.75 (2H, deuterium exchangeable, OH), 3.42 (1H, doublet, $J_{2,3}$=5 Hz, C_2–H), 3.45 (3H, singlet, OCH_3), 3.72 (1H, quartet, $J_{2,3}$=5 Hz, $J_{3,4}$= 3 Hz, C_3–H), 4.31 (1H, doublet, $J_{3,4}$=3 Hz, C_4–H), 5.05 (1H, singlet, C_1–H).

Methyl 4,6-Di-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-allopyranoside (**1b**)——Benzoylation of **1a** with benzoyl chloride in pyridine in the usual way gave **1b** in 62.5% yield, mp 121—122°, $[\alpha]_D^{22}$ +202.9° (c=0.31,

4) The $J_{3,4}$ value (4 Hz) of C_3–H in methyl 4,6-di-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-allopyranoside (**1b**) which contains a pyranoid ring is 4 Hz.
5) The $J_{3,4}$ value of C_3–H in the pyranoside derivatives, **2a** and its 4,6-di-O-benzoate (**2b**), is zero.
6) Melting points are uncorrected. Specific rotations were measured by automatic polarimeter (JASCO). PMR spectra were recorded at 100 MHz (JNM-MH 100) for solution in chloroform-d_3 unless otherwise stated. Infrared (IR) spectra were recorded with DS-701 (JASCO). Column chromatography was performed on 100 mesh silicic acid (Mallinchrodt Chem. Co.) with solvent systems as specified.

$CHCl_3$). ν_{max}^{KBr} 1725 cm^{-1} (C=O). PMR δ: 3.48 (3H, singlet, OCH_3), 3.64 (1H, quartet, $J_{1,2}=5$ Hz, $J_{2,3}=6$ Hz, C_2–H), 3.84 (1H, quartet, $J_{2,3}=6$ Hz, $J_{3,4}=4$ Hz, C_3–H), 5.26 (1H, doublet, $J_{1,2}=5$ Hz, C_1–H), 5.63 (1H, quartet, $J_{3,4}=4$ Hz, $J_{4,5}=9$ Hz, C_4–H). *Anal.* Calcd. for $C_{21}H_{20}O_6S$: C, 62.99; H, 5.04. Found: C, 63.00; H, 4.95.

Methyl 4,6-Di-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-mannopyranoside (**2b**)——Benzoylation of **2a** with benzoyl chloride in pyridine in the usual way gave colourless syrup of **2b** (79%), $[\alpha]_D^{21.5}$ +100.0° ($c=0.34$, $CHCl_3$). ν_{max}^{Neat} 1725 cm^{-1} (C=O). PMR δ: 3.15 (1H, doublet, $J_{2,3}=6$ Hz, C_2–H), 3.27 (1H, doublet, $J_{2,3}=6$ Hz, C_3–H), 3.54 (3H, singlet, OCH_3), 5.18 (1H, singlet, C_1–H), 5.48 (1H, doublet, $J_{4,5}=8$ Hz, C_4–H). *Anal.* Calcd. for $C_{21}H_{20}O_6S$: C, 62.99; H, 5.04. Found: C, 63.00; H, 5.25.

Methyl 5,6-Di-O-benzoyl-2,3-dideoxy-2,3-epithio-β-D-allofuranoside (**3b**)——Benzoylation of **3a** with benzoyl chloride in pyridine in the usual way gave colourless syrup of **3b** (85.4%), $[\alpha]_D^{20}$ −103.3° ($c=0.48$, $CHCl_3$). ν_{max}^{Neat} 1725 cm^{-1} (C=O). PMR (dimethylsulphoxide-d_6) δ: 3.44 (1H, doublet, $J_{2,3}=4.5$ Hz, C_2–H), 3.50 (3H, singlet, OCH_3), 3.62 (1H, doublet, $J_{2,3}=4.5$ Hz, C_3–H), 4.60 (1H, doublet, $J_{4,5}=9$ Hz, C_4–H), 4.62 (1H, quartet, $J_{5,6}=5$ Hz, $J_{6,6'}=12.6$ Hz, C_6–H), 4.92 (1H, quartet, $J_{5,6}=3$ Hz, $J_{6,6'}=12.6$ Hz, C_6–H′), 5.58 (1H, octet, $J_{4,5}=9$ Hz, $J_{5,6}=5$ Hz, $J_{5,6'}=3$ Hz, C_5–H). *Anal.* Calcd. for $C_{21}H_{20}O_6S$: C, 62.99; H, 5.04. Found: C, 62.93; H, 5.24.

Methyl 5,6-Di-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-mannofuranoside (**4b**)——Benzoylation of **4a** with benzoyl chloride in pyridine in the usual way gave colourless syrup of **4b** (83.0%), $[\alpha]_D^{21.5}$ +38.9° ($c=0.25$, $CHCl_3$). ν_{max}^{Neat} 1725 cm^{-1} (C=O). PMR δ: 3.35 (1H, doublet, $J_{2,3}=5$ Hz, C_2–H), 3.34 (3H, singlet, OCH_3), 3.62 (1H, quartet, $J_{2,3}=5$ Hz, $J_{3,4}=3$ Hz, C_3–H), 4.70 (1H, quartet, $J_{5,6}=5.2$ Hz, $J_{6,6'}=12$ Hz, C_6–H), 4.72 (1H, quartet, $J_{3,4}=3$ Hz, $J_{4,5}=8$ Hz, C_4–H), 4.88 (1H, quartet, $J_{5,6'}=3$ Hz, $J_{6,6'}=12$ Hz, C_6–H′), 5.09 (1H, singlet, C_1–H), 5.54 (1H, heptet, $J_{4,5}=8$ Hz, $J_{5,6}=5.2$ Hz, $J_{5,6'}=3$ Hz, C_5–H). *Anal.* Calcd. for $C_{21}H_{20}O_6S$: C, 62.99; H, 5.04. Found: C, 62.88; H, 5.18.

2,3-Dideoxy-2,3-epithio-β-D-allofuranose (**3c**)——a) A mixture of **1a** (1.5 g) and Amberlite CG-120(H^+) (4.5 g) in H_2O (150 ml) was stirred for 10 hr at 55°. After removal of the resin by filtration, the filtrate was evaporated *in vacuo* to dryness. The residue was chromatographed eluting with $CHCl_3$ gave crystalline **3c**. Recrystallization from acetone–pet. ether gave 1.065 g (76.6%) of colourless needles, mp 98—99°, $[\alpha]_D^{21}$ −18.4° (−32.0° after 24 hr, −42.8° after 48 hr) ($c=1.0$, MeOH) (equilibrium value). PMR (dimethyl sulphoxide-d_6) δ: 3.41 (1H, doublet, $J_{2,3}=5$ Hz, C_2–H), 3.79 (1H, doublet, $J_{2,3}=5$ Hz, C_3–H), 5.31 (1H, doublet collapsed into a singlet on addition of D_2O, $J=5.2$ Hz, C_1–H), 6.69 (1H, doublet, deuterium exchangeable, $J=5.2$ Hz, C_1–OH). *Anal.* Calcd. for $C_6H_{10}O_4S$: C, 40.45; H, 5.66. Found: C, 40.55; H, 5.62.

b) Under the similar way described above, **3a** (1.5 g) gave 1.077 g (77.7%) of **3c**.

1,5,6,-Tri-O-acetyl-2,3-dideoxy-2,3-epithio-β-D-allofuranose (**3d**)——Acetylation of **3c** with acetic anhydride in pyridine in the usual way gave colourless syrup of **3d** (65.3%), $[\alpha]_D^{25}$ −22.9° ($c=1.1$, $CHCl_3$). ν_{max}^{Neat} 1750 cm^{-1} (C=O). PMR (dimethylsulphoxide-d_6) δ: 3.09 (1H, doublet, $J_{2,3}=4.5$ Hz, C_2–H), 3.74 (1H, doublet, $J_{2,3}=4.5$ Hz, C_3–H), 4.03 (1H, quartet, $J_{5,6}=6$ Hz, $J_{6,6'}=12$ Hz, C_6–H), 4.36 (1H, doublet, $J_{4,5}=8$ Hz, C_4–H), 4.42 (1H, quartet, $J_{5,6'}=3$ Hz, $J_{6,6'}=12$ Hz, C_6–H′), 5.06 (1H, octet, $J_{4,5}=8$ Hz, $J_{5,6}$ 6 Hz, $J_{5,6'}=3$ Hz, C_5–H), 6.42 (1H, singlet, C_1–H). *Anal.* Calcd. for $C_{12}H_{16}O_7S$: C, 47.37; H, 5.30. Found: C, 47.29; H, 5.51.

Methyl Glycosidation of 3c with Amberlite CG-120(H^+)——A mixture of **3c** (28 mg) and Amberlite CG-120(H^+) (160 mg) in dry MeOH (5 ml) was stirred for 5 hr at 55°. After removal of the resin by filtration, the filtrate was evaporated in vacuo to dryness. The crystalline residue was recrystallized from $CHCl_3$ to afford 38.6 mg (59%) of **3a**, mp 134.5°, which was identified by direct comparison of mp and IR with those of an authentic sample of **3a**.

2,3-Dideoxy-2,3-epithio-α-D-mannofuranose (**4c**)——a) A mixture of **2a** (1.5 g) and Amberlite CG-120(H^+) (4.5 g) in H_2O (150 ml) was stirred for 16 hr at 55°. Work up as described previously gave colourless syrup, 914.4 mg (65.5%), of **4c**, $[\alpha]_D^{29.5}$ −72.5° ($c=0.6$, MeOH). PMR (dimethylsulphoxide-d_6) δ: 3.39 (1H, doublet, $J_{2,3}=5$ Hz, C_2–H), 3.74 (1H, quartet, $J_{2,3}=5$ Hz, $J_{3,4}=3$ Hz, C_3–H), 4.14 (1H, quartet, $J_{3,4}=3$ Hz, $J_{4,5}=8$ Hz, C_4–H), 5.24 (1H, doublet collapsed into a singlet on addition of D_2O, $J=6$ Hz, C_1–H), 6.63 (1H, doublet, deuterium exchangeable, $J=6$ Hz, C_1–OH). *Anal.* Calcd. for $C_6H_{10}O_4S$: C, 40.45; H, 5.66. Found: C, 40.74; H, 5.76.

b) In a similar way described above, **4a** (64.7 mg) gave 44.7 mg (74.3%) of **4c**.

1,5,6-Tri-O-benzoyl-2,3-dideoxy-2,3-epithio-α-D-mannofuranose (**4d**)——Benzoylation of **4c** with benzoyl chloride in pyridine in the usual way gave colourless syrup of **4d** (65.7%), $[\alpha]_D^{20}$ −20.7° ($c=0.63$, $CHCl_3$). ν_{max}^{Neat} 1725 cm^{-1} (C=O). PMR δ: 3.64 (1H, doublet, $J_{2,3}=5$ Hz, C_2–H), 3.76 (1H, quartet, $J_{2,3}=5$ Hz, $J_{3,4}=3$ Hz, C_3–H), 4.60 (1H, quartet, $J_{5,6}=5$ Hz, $J_{6,6'}=12$ Hz, C_6–H), 4.90 (1H, quartet, $J_{5,6'}=2.5$ Hz, $J_{6,6'}=12$ Hz, C_6–H′), 4.92 (1H, quartet, $J_{3,4}=3$ Hz, $J_{4,5}=8$ Hz, C_4–H), 5.57 (1H, heptet, $J_{4,5}=8$ Hz, $J_{5,6}=5$ Hz, $J_{5,6'}=2.5$ Hz, C_5–H), 6.62 (1H, singlet, C_1–H). *Anal.* Calcd. for $C_{27}H_{22}O_7S$: C, 66.12; H, 4.52. Found: C, 66.23; H, 4.57.

Methyl Glycosidation of 4c with Amberlite CG-120(H^+)——A mixture of **4c** (102 mg) and Amberlite CG-120(H^+) (310 mg) in dry MeOH (10 ml) was stirred for 5 hr at 55°. After removal of the resin by filtration, the filtrate was evaporated *in vacuo* to dryness. The residue was purified by chromatography ($CHCl_3$: AcOEt=1:1) to give crystals, 71.6 mg (71%), of **4a**, mp 113.5—116.5°. Recrystallization from $CHCl_3$

gave colourless needles, mp 116.5—117.5°, which was identified by direct comparison of mp and IR with those of an authentic sample of **4a.**

Acknowledgement The authors express their gratitude to the staffs of the analytical section of this Faculty for elemental analyses, and IR and PMR spectral measurements.

[Chem. Pharm. Bull. 25(5)1143—1146(1977)]

UDC 547.466.1.04 : 541.632

An Improved Attachment of N-Protected Amino Acid and Peptide to Chloromethylated Polystyrene-divinylbenzene Resin[1)]

KENJI SUZUKI and NOBUYOSHI ENDO

Tohoku College of Pharmacy[2)]

(Received August 18, 1976)

N-Protected amino acyl- or peptidyl-resin was prepared in good yield without racemization, when about a half equivalent amount of 1,5-diaza-bicyclo[4,3,0]nonene-5 (DBN) or 1,8-diaza-bicyclo[5,4,0]undecene-7 (DBU) salt of N-protected amino acid or peptide was allowed to react with chloromethylated polystyrene–2%-divinylbenzene resin (chlorine content 0.66 millimole per gram) at 50° for 28 hours. The unchanged chloromethyl group was acetylated quantitatively by the reaction with large excess of DBN salt of acetic acid.

Keywords——ester bond formation; 1,5-diaza-bicyclo[4,3,0]nonene-5; 1,8-diaza-bicyclo[5,4,0]undecene-7; N-protected amino acids; N-protected peptides; chloromethylated polystyrene–2%-divinylbenzene; racemization test

In solid phase peptide synthesis[3)] the most widely used starting material is a Boc-amino acid bound *via* a benzyl ester linkage to an insoluble copolymer of styrene and divinylbenzene (Boc-amino acyl-resin). Among many procedures for the preparation of Boc-amino acyl-resin investigated so far, it has been reported that the reaction of chloromethylated polystyrene-co-1%-divinylbenzene resin with the cesium salts of Boc-amino acid give Boc-amino acyl-resin in good yield.[4)] However, the procedure did not always give satisfactory result.[5)] On the other hand, for the synthetic strategy of fragment condensation on the solid support, it is required the attachment of large peptide to the solid support in good yield without racemization,[6)] although this has not been extensively tested.

The present communication deals with an improved attachment of N-protected amino acid and peptide to the solid support. As a basic catalyst, 1,5-diaza-bicyclo[4,3,0]nonene-5 (DBN)[7)] or 1,8-diaza-bicyclo[5,4,0]undecene-7 (DBU)[8)] was used in the reaction of chloromethylated polystyrene–2%-divinylbenzene resin (chlorine content 0.66 millimole or 1.10 millimole per gram) with Boc-amino acid or peptide in dimethylformamide (DMF). When

1) Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemical Nomenclature: *Biochemistry*, **11**, 1726 (1972). Other abbreviations: HOBt=1-hydroxybenztriazole, DCC=dicyclohexylcarbodiimide. The -resin represents ester bond derived from N-protected amino acid or peptide with chloromethylated polystyrene 2% divinylbenzene.
2) Locations: *Komatsushima, Sendai 983, Japan.*
3) R.B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
4) F.B. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
5) J. Burton, K. Poulsen and E. Haber, *Biochemistry*, **14**, 3892 (1975).
6) F. Waygand and U. Ragnarsson, *Z. Naturforsch.*, **21b**, 1141 (1966).
7) H. Oediger, H.J. Kabbe, Fr. Möller and K. Eiter, *Chem. Ber.*, **99**, 2012 (1966).
8) H. Oediger and Fr. Möller, *Angew. Chem. Internat. Ed.*, **6**, 76 (1967).