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PMR INVESTIGATION INTO THE STRUCTURE OF SOME N-SUBSTITUTED 1-AMINO-1-DEOXY-D-FRUCTOSES (AMADORI REARRANGEMENT PRODUCTS)

EVIDENCE FOR A PREFERENTIAL CONFORMATION IN SOLUTION

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Abstract—The PMR spectra at 220 MHz of some Amadori rearrangement products deduced from D-glucose with *p*-toluidine (1), N-methylphenylamine (2), di-butylamine (4), piperdine (5), and morpholine (6) have been studied in detail.

Compounds 1–6 appear to exist in solution predominantly as an equilibrium mixture of the furanose and pyranose ring. The pyranose ring occurs exclusively in the β(D)-$^2C_5$-conformation (corresponds to Reeves 1C-conformation). The furanose ring probably exists as a mixture of both the β- and α-anomer, in which the β-anomer is favoured.

INTRODUCTION

An Amadori compound[1] arises from a complete conversion of an N-substituted aldosylamine in the presence of an acid to an N-substituted 1 - amino - 1 - deoxy - 2 - ketose (I) or a hemiacetal ring structure derived from it (II, III) (see Scheme 1).

Generally the presence or absence of a CO absorption band in the IR spectra is used to assign the keto structure (I)[2–5] or the cyclic forms (II, III),[4–8] respectively. As far as the cyclic structures are concerned, no further spectroscopical evidence is given for either the 5-II or the 6-membered ring-III structure. Usually it is assumed[6] that the 6-membered ring is more stable.

The first assignment of ring size to an Amadori compound is given by Kuhn *et al.*[9] by chemical means. They demonstrate that 1,3,4,5 - tetra - O - methyl - β - D - fructopyranose can be formed from the Amadori compound 1 - deoxy - p - tolylamino - D - fructose.

It should be possible to distinguish between structures I, II and III on the basis of the vicinal and geminal coupling constants. For instance, the magnitude of the geminal coupling constant of the methylene group at C atom 1′ in structure I ($J \simeq 17$–19 Hz)[10,11] will differ considerably from that of the corresponding group in II or III ($J \simeq 11$–13 Hz). From the magnitude of the vicinal coupling constant we may even be able to deduce the conformation of the ring in structure III.

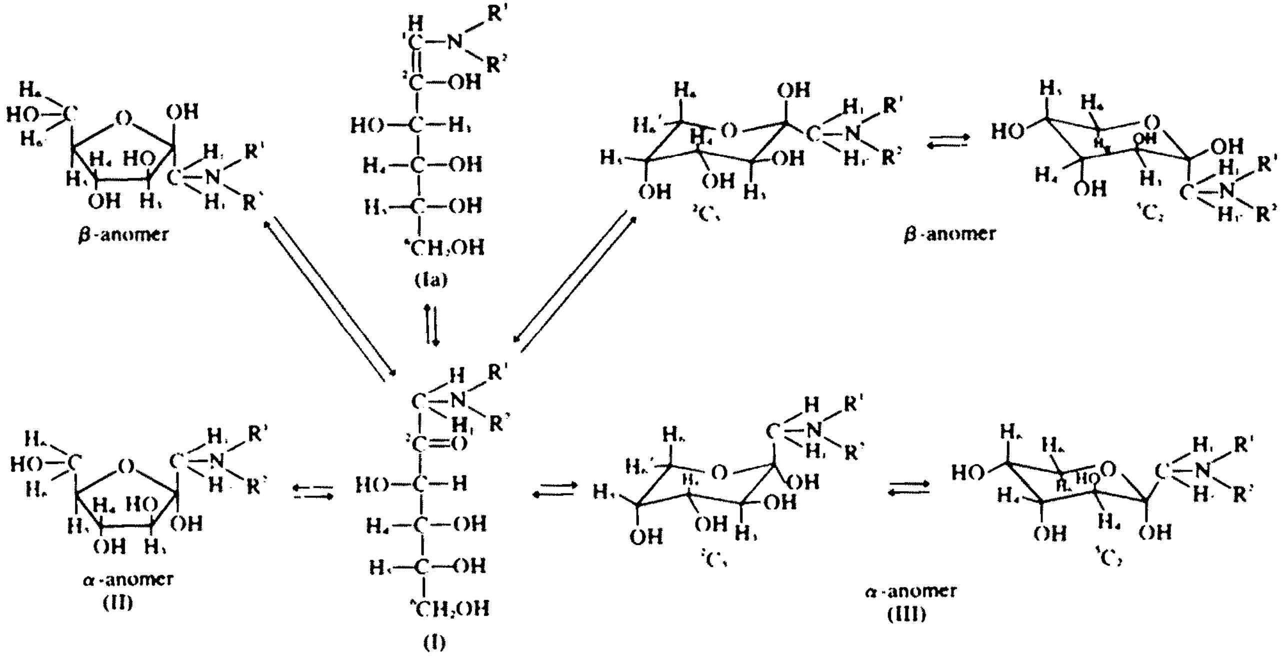
In order to present direct spectroscopical evidence for structures I, II or III, and the position of the hemiacetal OH-group under a given set of conditions, we studied the PMR spectra at 220 MHz of the following Amadori compounds (Scheme 1):

1: $R' = \phi CH_3$, $R^2 = H$ (Ref 7)
2: $R^1 = \phi$, $R^2 = CH_3$ (Ref 5)
3: $R^1 = R^2 = \phi CH_2$ (Ref 12)
4: $R^1 = R^2 = CH_3(CH_2)_3$ (Experimental)
5: $R^1 + R^2 = c(C_5H_{10})$ (Ref 5)
6: $R^1 + R^2 = c(C_4H_8O)$ (Ref 12).

In addition we examined the PMR spectrum of β - D - fructose (7) as reference compound.

RESULTS AND DISCUSSION

Analysis of the PMR spectra. Examination of the PMR spectra of 1–6, recorded in 5-deuteropyridine (pyr-d_5) shows that all the compounds, except 3, consist of a mixture of at least two components. As 3 occurs as a single component, the assignment of the signals in its spectrum is facilitated. The signals of H_1 and H'_1 appear as two doublets at $\delta = 3{\cdot}50$ and $3{\cdot}88$ ppm (J gem = $13{\cdot}5$ Hz). The doublet at $\delta = 4{\cdot}87$ ($J = 10{\cdot}0$ Hz) should be assigned to proton H_3 (in each of the structures I, II or III as given in Scheme 1, H_3 is coupled only with H_4). On account of the observed spacings of 10·0 and 3·0 Hz, the signal at $\delta = 4{\cdot}95$ ppm arises from H_4. Among the remaining signals the multiplet at $\delta = 4{\cdot}75$ which is not well resolved can be attributed to H_5, and the doublet of doublets at $\delta = 4{\cdot}49$ ($J = 12{\cdot}0$ and $1{\cdot}0$ Hz) and at $\delta = 4{\cdot}83$ ppm ($J = 12{\cdot}0$ and $1{\cdot}0$ Hz) to H'_6 (axial) and H'_6 (equatorial) respectively.



SCHEME 1. Formation of Amadori compounds. (The symbols $^2C_5$ and $^5C_2$ are used to describe the two chair conformations of a pyranose of the D-series, according to Ref 2; these symbols correspond to the Reeves 1C and C1 notation respectively.)

Table 1. Chemical shifts δ (in ppm) of the protons of the 1-alkylamino-β-D-arabino-hexulo-pyranose structure, the major component in compounds **1–6**

Compound	R^1	R^2	Solvent	δH_1	$\delta H_{1'}$	δH_3	δH_4	δH_5	δH_6	$\delta H_{6'}$	Others
1	$pCH_3\phi$	H	pyr-d_5	3·81(dd)[a]	4·03(dd)[a]	4·73(d)	4·66(dd)	4·39(m)	4·13(dd)	4·51(dd)	2·15(s); 5·37(br.t); 6·73(d); 6·93(d)
2	ϕ	CH_3	pyr-d_5	3·77(d)[b]	3·98(d)[b]	4·50(d)	4·65(dd)	4·38(m)	4·13(dd)	4·48(dd)	3·00(s), or 3·05(s); 7·43(m)
3	ϕCH_2	ϕCH_2	pyr-d_5	3·50(d)	3·88(d)	4·87(d)	4·95(dd)	4·75(m)	4·49(dd)	4·83(dd)	4·14(d); 4·52(d); 7·25(m); 7·50(m)
4	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$	pyr-d_5	2·60(d)	3·36(d)	4·33(d)	4·57(dd)	4·36(m)	4·05(dd)	4·40(dd)	0·82(t); 1·2–1·4(m); 2·4–2·7(m)
5	$c(C_5H_{10})$		pyr-d_5	2·69(d)	3·18(d)	4·35(d)	4·56(dd)	4·33(m)	4·02(dd)	4·39(dd)	1·22(m); 1·39(m); 2·39(m); 2·50(m)
			D_2O			3·79(d)	3·86(dd)	3·97(m)	3·69(dd)	4·02(dd)	1·44(m); 1·59(m); 2·6–2·7(m)
6	$c(C_4H_8O)$		pyr-d_5	2·78(d)	3·17(d)	4·44(d)	4·58(dd)	4·36(m)	4·04(dd)	4·41(dd)	2·53(m), 2·86(m); 3·60(t)
			D_2O	2·70(s)[d]		3·69(d)	3·86(dd)	3·96(m)	3·63(dd)	4·01(dd)	2·6–2·8(m); 3·76(t)

[a] Doublet of doublets collapse into two doublets after additions of a few drops of CD_3OD.

[b] Tentative assignment.

[c] Completely exchanged with deuterium.

[d] Partly exchanged with deuterium.

A similar analysis of the spectra of **1**, **2**, **4**, **5** and **6** in pyr-d_5 gave the results shown in Tables 1 and 2. From the striking similarities in coupling constants (Table 2) we conclude that the major components in these five compounds have the same structure as **3**. Comparison of the coupling constants of **5** and **6** (Table 2, values between brackets), in D_2O with those determined in pyr-d_5 indicates that in D_2O **5** and **6** also have the same structure as **3**.

The middle spectrum in Fig 1 is the PMR spectrum of **2** in pyr-d_5 in the low-field region. The signals due to the major component are assigned as indicated in the figure and the assignments are confirmed by spectrum simulation (lower spectrum).

The doublets at $\delta = 3{\cdot}80$ ($J = 15$ Hz) and at $\delta = 4{\cdot}19$ ppm ($J = 15$ Hz, partly overlapped) are tentatively assigned to H_1 and H_1', of the minor component in **2**. It should be remarked that this assignment may equally well be interchanged with the signals at $\delta = 3{\cdot}77$ and $\delta = 3{\cdot}98$ ppm. Proton H_3 and H_4 of the minor component can be easily recognized as a doublet of doublets at $\delta = 4{\cdot}71$ ppm ($J = 7{\cdot}5$ Hz) and at $\delta = 5{\cdot}07$ ppm ($J = 7{\cdot}0$ and $7{\cdot}5$ Hz) respectively. H_6 and H_6' appear as a doublet of doublets at $\delta = 4{\cdot}20$ ($J = 3{\cdot}0$ and 11 Hz) and at $\delta = 4{\cdot}28$ ppm ($J = 4{\cdot}0$ and 11 Hz). The signal of proton H_5 is obscured by signals of the major component. The upper spectrum in Fig 1 is the simulated spectrum of the minor component in **2** and confirms the assignment of the different signals.

The first-order chemical shifts and coupling con-

Table 2. First-order coupling constants (Hz) of the protons of the major component in compounds **1**–**6**, determined from the spectra recorded in pyridine-d_5 as solvent

Compound	J'_{11}	J_{34}	J_{45}	J_{56}	J'_{56}	J'_{66}
1	12·0[a]	10·0	3·0	1·5	1·0	12·0
2	15·0	10·0	3·0	1·5	1·5	12·0
3	13·5	10·0	3·0	1·5	1·0	12·0
4	13·0	10·0	3·0	1·5	1·5	12·0
5[b]	13·0	10·0(10·0)	3·5(3·5)	1·5(1·5)	1·5(1·0)	12·0(12·5)
6[b]	13·0	10·0(10·0)	3·0(3·5)	1·5(1·5)	1·0(1·0)	12·0(12·5)

[a] Coupling constants with the NH-proton were 3·0 and 7·0 Hz respectively.

[b] Values between brackets were determined from the spectra recorded in D_2O as solvent.

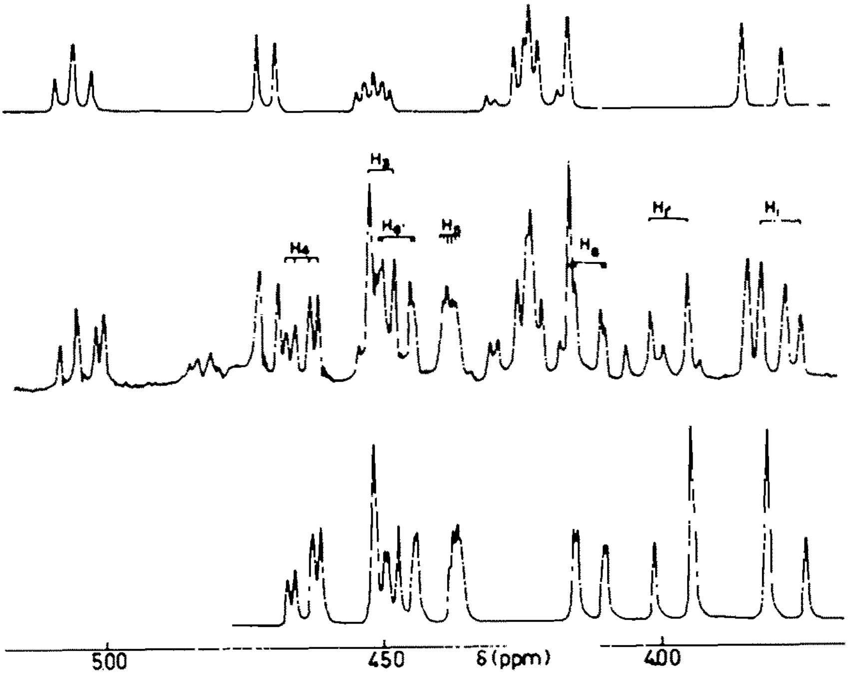


Fig 1. PMR spectrum of 1-deoxy-1-(N-methylanilino)-D-fructose (**2**) in pyridine-d_5 at 220 MHz (middle). Simulated spectrum of minor component in **2** (upper) and of major component in **2** (lower).

stants of the minor component in 1–6 are given in Tables 3 and 4.

Structural assignment. The magnitude of the geminal coupling constants of the methylene protons (H_1 and H'_1) next to the N atom, which varies between 12·0 and 15·0 Hz (Table 2), indicates that the major component in 1–6 occurs either in the furanose (2) or pyranose form (3) (Scheme 1).

Irrespective of whether the major component of 1–6 occurs as the β- or α-anomer, the values of the vicinal coupling constants given in Table 2 provide unequivocal evidence for the 6-membered ring structure in the D-$^2C_5$-conformation (Scheme 1). It is, however, reasonable to assume that all the alkylamino groups in 1–6 will preferably adopt an equatorial position (= β-anomer). The good correspondence in chemical shifts and coupling constants of 1–6 with those of β-D-fructose at equilibrium (Tables 5 and 6) confirms our assumption, as it has been proved[13] that D-fructose in solution exists predominantly in the more stable β-pyranoid form. The magnitude of the vicinal coupling constants of β-D-fructose (Table 6) clearly shows that the pyranose ring of β-D-fructose has the $^2C_5$-conformation (Reeves 1C conformation) in aqueous solution. The doublet at $\delta = 4{\cdot}07$ ppm ($J = 3{\cdot}5$ Hz) has been tentatively assigned to proton H_3 of β-D-fructofuranose.[13]

The values of $J_{11'}$ coupling constants and vicinal coupling constants (Table 4) for the minor component, together with the fact that the major component in 1–6 has the pyranose form, indicate that the minor component in 1–6 has the 5-membered ring structure 2 (Scheme 1).

Confirmatory evidence for this conclusion is presented by the absence of an absorption at

Table 3. Chemical shifts δ (in ppm) of the protons of the 1-alkylamino-α,β-D-arabino-hexulofuranose structure, the minor component in compounds 1–6, determined from the spectra recorded in pyridine-d_5 as solvent

Compound	δH_1	$\delta H'_1$	δH_3	δH_4	δH_5	δH_6	$\delta H'_6$	Others
1	3·77(d)	3·92(d)	4·85(d)	5·05(dd)	4·54(m)	4·21(dd)	4·28(dd)	2·17(s); 6·84(d); 6·98(d)
2	3·80(d)	4·19(d)	4·71(d)	5·07(dd)	4·51(m)	4·20(dd)	4·28(dd)	3·05(s) or 3·00(s); 7·51(m)
	[3·96(d)]*	[4·09(d)]*	[5·05(d)]*					[3·02(s); 3·15(s)]
3	—	—	—	—	—	—	—	—
4	2·94(d)	3·04(d)	4·58(d) [4·85(d)]*	5·00(dd)	4·53(m)	4·27(dd)	4·29(dd)	1·69(m)
5	2·77(d)	2·94(d)	4·54(d) [4·85(d)]*	4·94(dd)	4·50(m)	4·20(dd)	4·25(dd)	—
6	—	—	—	—	—	—	—	2·67(m)

*Signal has been tentatively attributed to the α-anomer.

Table 4. First-order coupling constants (Hz) of the protons of the minor component in compounds 1–6, determined from the spectra recorded in pyridine-d_5 as solvent

Compound	J'_{11}	J_{34}*	J_{45}	J_{56}	J'_{56}	J'_{66}
1	13·0	7·5	6·0	5·0	3·0	12·0
2	15·0(15·0)*	7·5(3·5)	7·0	3·0	4·0	11·0
3	—	—	—	—	—	—
4	14·0	7·0(3·0)	6·0	4·5	5·5	11·0
5	13·0	6·5(3·5)	6·0	4·5	5·5	11·5
6	—	—	—	—	—	—

*Values between brackets are tentatively assigned to the α-anomer.

Table 5. Chemical shifts δ (in ppm) of the protons of β-D-fructose (7) at equilibrium

Solvent	δH_1	$\delta H'_1$	δH_3	δH_4	δH_5	δH_6	$\delta H'_6$	Others
Pyr-d_5	4·2–4·3*		4·83(d)	4·66(dd)	4·38(m)	4·12(dd)	4·50(dd)	4·60(m); 5·16(d)
D_2O	3·52(d)	3·68(d)	3·76(dd)	3·86(dd)	3·95(m)	3·67(dd)	3·99(dd)	4·07(d)

*Not resolved.

Table 6. Coupling constants J (in Hz) of the protons of β-D-fructose (7)

Solvent	J_{11}	J_{34}*	J_{45}	J_{56}	J'_{56}	J'_{66}
Pyr-d_5	—	10·0 (7·0)	3·5	1·5	1·5	12·0
D_2O	12·0	10·0 (3·5)	3·0	1·5	1·0	12·5

*Values between brackets are tentatively assigned to β-anomer.

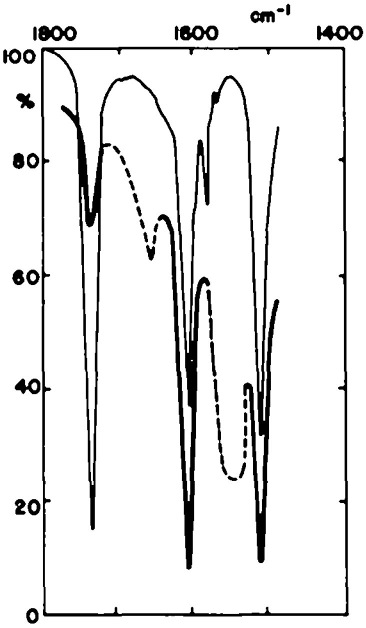


Fig 2. Infrared spectra of 1 - deoxy - 1 - (N - methylanilino) - D - fructose (2): KBr disk (thin line), in pyridine-d_5 (bold line).

1720 cm^{-1} in the IR spectra of 1 and 3–6 (pyr-d_5). Besides the doublet with a coupling constant of about 7·0 Hz, assigned to proton H_3 of the furanose form, the spectra of 2, 4 and 5 in pyr-d_5 also show a doublet with a lower intensity and a spacing of 3·5 Hz (e.g. the doublet at $\delta = 5·05$ ppm in the spectrum of 2 in Fig 1).

It is not unreasonable to assume that both the β- and α-anomer of the furanose form are present in solution and that the doublet at lowest field and with the lower intensity arises from proton H_3 of the α-anomer.[13]

In contrast to 1 and 3–6 in pyr-d_5, 2 in pyr-d_5 shows in its IR spectrum a small absorption at 1720 cm^{-1} (Fig 2) due to structure I (Scheme 1). The IR spectrum of 2 in KBr (Fig 2), however, clearly indicates that, in the solid state, 2 exists exclusively in the 1 - deoxy - 2 - ketose form[5] (I).

Assuming that the extinction coefficient of the CO group in the solid state is about the same as in pyr-d_5 solution, the amount of I can be estimated to be lower than 10%. This value agrees well with the amounts of I given in Table 7, in which the equilibrium compositions for compounds 1–6 are summarized. These values are obtained by measurement of the peak intensities in the NMR spectra.

Whatever the structure of the Amadori rearrangement products in the solid state may be I, II or III, in solution they evidently all exist in equilibrium with each other (Scheme 1), and the cyclic structures II or III are favoured. The complete exchangeability of the methylene protons H_1 and H'_1 of 5 and 6 by deuterium strongly supports such an equilibrium. Such an exchange takes only place *via* the enol form Ia (Scheme 1) of 1 - deoxy - 2 - ketose structure I. The position of the equilibrium varies somewhat, depending on the substituents on the amino group (Table 7) and the solvent (Table 8).

In more polar solvents the furanoid form of 4 is favoured (Table 8), probably because its polar groups are better distributed than those of the pyranoid form of 4 in such solvents.[14]

Table 7. Equilibrium composition (%) of I, II and III for compounds 1–6 in pyridine-d_5 (conc 0·2 M; temp 15°)

		II		III
Compound	I	β-anomer	α-anomer	β-anomer
1	—	20	5	75
2	5	39	8	48
3	—	—	—	100
4	—	26	11	63
5	—	25	13	62(100)*
6	—	30		70(100)*

*In D_2O solution.

Table 8. Influence of solvent on the equilibrium composition (%) of I, II and III for 4 (conc 0·2 M; temp 15°)

			II		III
Solvent	Dielectric constant	I	β-anomer	α-anomer	β-anomer
C_6D_6	2·3	—	10		90
$CDCl_3$	4·8	—	15	5	80
Pyr-d_5	12·7	—	26	11	63
CD_3COCD_3	20·7	—	28	17	55

CONCLUSIONS

1. The Amadori compounds **1**–**6** deduced from N-substituted D-isoglucosamines exist in solution predominantly as an equilibrium mixture of the furanose and the pyranose ring. The position of the equilibrium depends on the substituent on the amino group as well as the solvent.

2. The pyranose form occurs exclusively in the β (D) - $^{2}C_{5}$ - conformation (Reeves 1C-notation).

3. The furanose ring probably exists as both the β- and α-anomer, with the β-anomer being favored.

EXPERIMENTAL

Methods. The PMR spectra at 220 MHz were recorded with a Varian spectrometer.* Sodium 2,2,3,3, - tetradeutero - 3 - (trimethylsilyl) proprionate was used as the internal standard in D_2O soln. A Varian A 620 i computer was used for spectrum simulation.

Synthesis of 1 - *deoxy* - 1 - *di* - *n* - *butylamino* - D - *fructose.* A mixture of 99 g (0·5 mole) of α - D - glucose hydrate and 75 ml of triethylamine was placed in a 500 ml 3-necked flask fitted with a reflux condensor, a thermometer and a stirrer. 64·5 g (0·5 mole) of di - n - butylamine and 30 g (0·5 mole) of AcOH were added simultaneously to the vigorously stirred mixture. The warm mixture (45°) was then heated to 75°, and kept at that temp for 1 h. The mixture was then poured into 1 litre of a 1:1 mixture of EtOH and acetone. After being left to stand overnight at 0° the product was filtered off. Recrystallization from acetone yielded the pure 1 - deoxy - 1 - di - n - butylamino - D - fructose with m.p. 98°–99°, $[\alpha]_D^{20}$: − 85·8° (MeOH).

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REFERENCES

[1] L. M. J. Verstraeten, *Advan. Carbohydrate Chem.* **22**, 272 (1967)
[2] Anon., *J. C. S. Chem. Comm.* 505 (1973)
[3] E. F. L. J. Anet and T. M. Reynolds, *Austr. J. Chem.* **10**, 182 (1957); F. Micheel and A. Frowein, *Chem. Ber.* **90**, 1599 (1957); K. Heyns and W. Baltes, *Ibid.* **93**, 1616 (1960); K. Heyns, T. Chiemprasert and W. Baltes, *Ibid.* **101**, 2877 (1970)
[4] K. Heyns and W. Baltes, *Ibid.* **91**, 622 (1958); F. Micheel and G. Hagemann, *Ibid.* **92**, 2836 (1959)
[5] F. Weygand, H. Simon and R. von Ardenne, *Ibid.* **92**, 3117 (1959)
[6] E. F. L. J. Anet, *Austr. J. Chem.* **12**, 280 (1959)
[7] F. Micheel and W. Hühne, *Chem. Ber.* **93**, 2383 (1960)
[8] K. Heyns, H. Paulsen and H. Schroeder, *Tetrahedron* **13**, 247 (1961)
[9] R. Kuhn, G. Krüger and A. Seeliger, *Liebigs Ann.* **618**, 82 (1958)
[10] Y. L. Chow and C. J. Colón, *Can. J. Chem.* **45**, 2559 (1967)
[11] S. J. Angyal and K. James, *Austr. J Chem.* **23**, 1225 (1970)
[12] J. E. Hodge and B. E. Fisher, *Methods in Carbohydrate Chemistry* Vol. II, p. 99. Academic Press Inc., New York (1963)
[13] D. Doddrell and A. Allerhand, *J. Am. Chem. Soc.* **93**, 2779 (1971)
[14] W. Pigman and H. S. Isbel, *Advan. Carbohydrate Chem.* **23**, 33 (1968)