Carbohydrate Research, 229 (1992) 307–322
Elsevier Science Publishers B.V., Amsterdam

Influence of malondialdehyde on the Maillard degradation of Amadori compounds *,†

Antonio Gómez-Sánchez, Isidro Hermosín and Inés Maya

Instituto de la Grasa y sus Derivados, C.S.I.C., and Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado de Correos No. 1078, 41012 Seville (Spain)

(Received July 6th, 1991; accepted November 5th, 1991)

ABSTRACT

1-Amino-1-deoxy-D-fructose (**7**), and its *N*-butyl derivative (**8**), reacted with malondialdehyde to yield the 1-deoxy-1-(3-oxo-1-propenylamino)-D-fructoses **9** and **10**, respectively. Small proportions of 4-methyl-1,4-dihydro-3,5-pyridinedicarbaldehyde (**12**) and 1-deoxy-1-(3,5-diformyl-4-methyl-1,4-dihydropyridin-1-yl)-D-fructose (**14**) were also formed in the reaction with **7**, and of 1-butyl-4-methyl-1,4-dihydro-3,5-pyridinedicarbaldehyde (**13**) in the reaction with **8**. The reaction of **7** with methylmalondialdehyde afforded 1-deoxy-1-(2-methyl-3-oxo-1-propenylamino)-D-fructose (**11**). The enaminals **9** and **10** cyclised, in neutral or weakly alkaline aqueous solution, into a mixture of 4-(D-*arabino*-tetritol-1-yl)-3-pyrrolecarbaldehyde (**15** and **17**, respectively) and 3-(D-*arabino*-tetritol-1-yl)-4-pyridone (**19** and **21**, respectively). Small proportions of 4-(hydroxymethyl)-3-pyrrolecarbaldehyde (**16**) and 3-(hydroxymethyl)-4-pyridone (**20**) were also formed in the cyclisation of **9**, and of 1-butyl-4-(α,β-D-erythrofuranosyl)-3-pyrrolecarbaldehyde (**18**α,β) in the cyclisation of **10**. All of the heterocyclic compounds were unstable.

INTRODUCTION

The non-enzymic formation of brown pigments in food and in living matter (the Maillard reaction) is generally considered to encompass solely reactions of amino acids, peptides, and proteins with reducing sugars[2]. This view is a simplification, as other biocomponents may also participate in the process. The importance of lipid interaction in the Maillard reaction for the formation of flavour has been underlined[3], and oxidised lipids produce browning in fish muscle and in lysine–ribose–lipid model systems[4]. Malondialdehyde (MDA), which is distributed widely in mammalian tissues and in lipid-rich foods as a product of the peroxidation of polyunsaturated lipids, has been associated with the cross-linking of proteins[5], cell aging[6], and the deterioration of food[7]. The fluorescent lipofuscin pigments that

Correspondence to: Professor A. Gómez-Sánchez, Instituto de la Grasa y sus Derivados, C.S.I.C., and Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Apartado de Correos No. 1078, 41012 Seville, Spain.

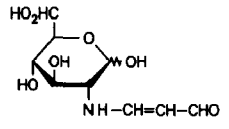
* Studies of the Maillard reaction, Part II. For Part I, see ref. 1.

† Dedicated to Professor Sidney Alan Barker on the occasion of his 65th birthday.

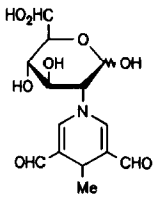
accumulate in aging organisms as a result of peroxidation of lipids may derive from the interaction of MDA with bioamines[6,8]. MDA reacts readily[1] with 2-amino-2-deoxy-D-glucose, producing 2-deoxy-2-(3-oxo-1-propenylamino)-D-glucose (**1**) and the dihydropyridine derivative **2**, and **1** can cyclise to give a mixture of the 3-pyrrolecarbaldehydes **3**–**5**.

When a solution of the sodium salt (NaMDA) of MDA is stored at room temperature and pH 5–7, cleavage of the dialdehyde takes place, yielding acetaldehyde that reacts with the excess of MDA to yield 2,4-di(hydroxymethylene)-3-methylglutaraldehyde[9] (**6**). Compound **6** is usually present in aqueous solutions of MDA at pH 5–6, and in crude preparations of NaMDA. The formation of **2** in the reaction of NaMDA with 2-amino-2-deoxy-D-glucose can be explained in these terms[1].

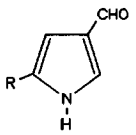
Glycosylamino acids and their Amadori rearrangement products, the first intermediates of the Maillard reaction, could co-exist and react with MDA, and the highly reactive products derived therefrom[9], in vivo and in partially oxidised lipid-rich foods. The products may have the physical properties typical of the Maillard products, thus contributing to browning. In order to gain an insight into the influence of oxidised lipids on the non-enzymic browning and in related processes, the reactions of MDA with 1-amino-1-deoxy-D-fructose (**7**) and with its *N*-butyl derivative (**8**) have been investigated, as simple models for the Amadori compounds present in food and in living systems.

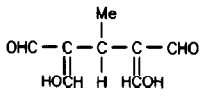


1

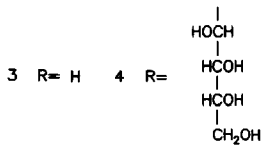


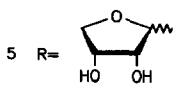
2





6





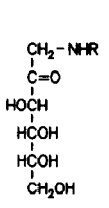
RESULTS AND DISCUSSION

1-Amino-1-deoxy-D-fructose (**7**) and 1-butylamino-1-deoxy-D-fructose (**8**), used as the acetate and oxalate, respectively, reacted with NaMDA (1 equiv) in water at room temperature to afford the enaminals **9** and **10**. Monitoring of the reaction by TLC and UV spectroscopy revealed that the rate was pH-dependent, being fastest at pH 3–4. Under these conditions, the aminoketoses were transformed almost quantitatively, and the enaminals were formed in yields of > 95%. The formation of by-products was also observed. Thus, the 1,4-dihydropyridines **12** and **14** were formed from **7**, and 1-butyl-1,4-dihydropyridine (**13**) from **8**. TLC enabled the isolation of hygroscopic and amorphous **9** (60%) and **10** (60%), and crystalline **12** (< 1%), **13** (< 1%), and **14** (4%).

The similar reaction of the acetate of **7** with the sodium salt of methylmalondialdehyde yielded (70% after chromatography) 1-deoxy-1-(2-methyl-3-oxo-1-propenylamino)-D-fructose (**11**).

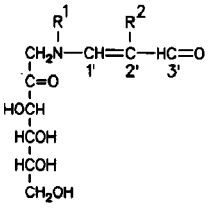
The structures of **9–11** were established on the basis of their analytical data and spectral properties. The compounds exhibited UV absorptions very similar to those of *N*-mono- and *N*-di-alkyl-3-aminoacroleins[10] and of **1**[1], and they had IR bands for C=O and C=C (or C=C + N–H) anticipated[1,11] for the electron-delocalised N–C=C–C=O (or H–N–C=C–C=O) system.

Due to the restricted rotations present in the 3-oxopropenyl group and the tautomerism of the D-fructose moiety (see Scheme 1), **9–11** can exist in several isomeric forms and this was clearly revealed by the NMR spectra (Table I). Thus, **9** existed in D_2O as an equilibrium mixture of six isomeric forms, two of which preponderated. The resonances of H-1′ and H-3′ each appeared as two doublets, of approximately the same intensity, and H-2′ as two doublets of doublets of



7 R= H

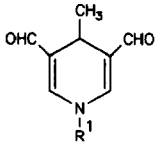
8 R= Bu



9 $R^1 = R^2 = H$

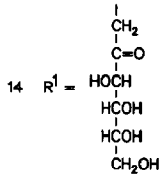
10 R^1 = Bu, R^2 = H

11 R^1 = H, R^2 = Me

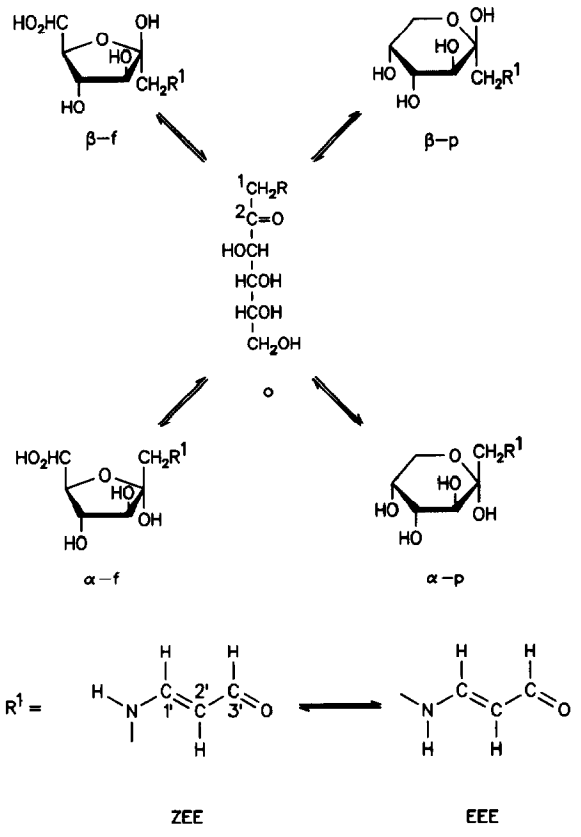


12 R^1 = H

13 R^1 = Bu



14 R^1 =



Scheme 1. Equilibria for 1-deoxy-1-(3-oxo-1-propenylamino)-D-fructose (**9**) in D_2O solution.

almost the same intensity. Accordingly, C-1′,2′,3′ each gave two signals with the anticipated δ values. These signals overlapped other weaker signals due to the same nuclei of the minor isomers. The $J_{1',2'}$ (12.1–12.7 Hz) and $J_{2',3'}$ (9.3–9.6 Hz) values, by analogy with those observed[1] for **1** and for simple 3-alkylamino-acroleins[10], indicated that the –CH=CH–CH=O group had the *EE* structure. Consequently, the isomers should differ in the conformation around the N–C-1′ bond and/or the geometry of the fructose moiety (Scheme 1). The acyclic tautomer (*o* form; Scheme 1) is rarely observed[12–16] and has never been observed in enaminones derived from 1-amino-1-deoxy-D-fructose[16], the *α-p* form is rare and is observed only in small proportions[12–16], and the *β-p* form preponderates (> 65%) in water[12–16]. Therefore, it is assumed that the two major isomers of **9** are the *Z* and *E* rotamers of the N–C-1′ bond of the *β-p* form. The complete geometry of these isomers should then be *β-p,ZEE* and *β-p,EEE* (Scheme 1). The assignment of the signals due to each of the isomers was made by considering the γ-deshielding effect[17] produced by the sugar moiety on H-2′ in the *ZEE* conformation. Thus, of the two doublets due to H-2′, that at lower field (δ 5.34) was assigned to the *ZEE* form, and the other (δ 5.30) to the *EEE* form. The two isomers were in the

TABLE I

NMR data[a] for a solution of **9** in D_2O (δ in ppm, J in Hz)

Atom	*β-p-ZEE*	*β-p-EEE*	*β-f-ZEE*	*β-f-EEE*	*α-f-ZEE*	*α-f-EEE*
H-1′	7.45d ($J_{1',2'}$ 12.7)	7.34d ($J_{1',2'}$ 12.1)				
H-2′	5.34dd ($J_{2',3'}$ 9.3)	5.30dd ($J_{2',3'}$ 9.6)				
H-3′	8.62d	8.55d				
Sugar moiety protons	3.2–4.0m					
C-1′	163.3	167.4	163.3	167.3	163.3	167.6
C-2′	101.0	102.8	101.2	102.8	101.0	102.8
C-3′	193.4	192.3	193.4	192.2	193.3	–
C-1	49.5	54.7	48.5	53.6	48.2	53.3
C-2	98.4	98.3	101.2	101.2	104.7	105.2
C-3	70.4	70.4	77.2	77.4	82.2	82.6
C-4	69.9	69.9	75.1	75.1	76.7	77.1
C-5	69.4	69.3	81.7	81.4	82.8	82.8
C-6	64.4	64.4	63.1	63.1	61.7	61.9

[a] At 200 MHz (^1H) and at 50.2 MHz (^{13}C).

ratio ~ 11:9. The assignments of the H-1′ and H-3′ signals of each isomer were made on the basis of their relative intensities.

The ^{13}C-NMR spectrum of **9** confirmed the above conclusions and could be analysed as three overlapping spectra due to the *β-p*, *β-f*, and *α-f* forms in the ratios 72:16:12 by consideration of the chemical shifts of C-2/6[12–16]. Each of these spectra was split into two very similar sub-spectra corresponding to the conformers *ZEE* and *EEE* in the ratio 10:8. Splitting of the signals due to these two isomers has been observed in the ^{13}C-NMR spectrum of **1**, and, as in this compound, the assignment of the *E* and *Z* geometry around the N–C-1′ bond in **9** was made on the basis of the γ-shielding effect[17] produced by the sugar moiety on C-2′ in the *Z* geometry. For the *ZEE* isomers, the resonances of C-2′ were in the range 101.0–101.2 ppm, whereas, for the *EEE* isomers, the C-2′ resonance was at 102.8 ppm. The two groups of isomers also differed in the chemical shifts of the resonances of C-1′, C-3′, and C-1. Similarly, the NMR spectra of **10** (Table II) in D_2O revealed the *ZEE,β-p*, *EEE,β-p*, *ZEE,β-f*, *EEE,β-f*, *ZEE,α-f*, and *EEE,α-f* isomers in the ratios ~ 31:49:7:17:2:4.

The NMR spectra of **11** (Table III) were simpler and revealed three isomers, in the ratios ~ 74:17:9; consideration of the δ values and comparison with the spectra of **9**, indicated that the isomers were the *β-p*, *β-f*, and *α-f* forms, each with the *EEE* conformation. Thus, the introduction of a Me group at C-2′ strongly destabilises the *ZEE* conformation.

The 1,4-dihydropyridines **12** and **13** are obtained[9,18] by reaction of the monosodium salt of **6** with ammonium acetate and butylamine, respectively[9]. Similarly, **14** was obtained (30%) by reaction of the monosodium salt of **6** with the

TABLE II

NMR data[a] for a solution of **10** in D_2O[b] (δ in ppm, J in Hz)

Atom	*β-p-ZEE*	*β-p-EEE*	*β-f-ZEE*	*β-f-EEE*	*α-f-ZEE*	*α-f-EEE*	*α-p-ZEE*	*α-p-EEE*
H-1′	7.45d ($J_{1',2'}$ 12.3)	7.34d ($J_{1',2'}$ 12.4)						
H-2′	5.40dd ($J_{2',3'}$ 9.4)	5.25dd ($J_{2',3'}$ 9.4)						
Sugar moiety protons	3.2–4.1m							
CH_2	3.2–4.1m							
CH_2	1.41–1.55m							
CH_2	1.10–1.29m							
CH_3	0.79t (J 7.3)							
C-1′	166.8	166.4	166.2	165.9				
					(161.6–162.6)			
C-2′		101.6	101.9					
					(100.7–101.1)			

C-3′			192.1–192.3					
					(188.2–188.6)			
C-1	58.1	60.8	57.6	60.2				
	(56.2)	(59.9)	(55.5)	(59.0)	(55.6)	(58.1)		(59.3)
C-2	100.0	99.3	102.8	102.2		105.5		
		(98.7)	(102.5)	(102.0)		(104.6)		(97.5)
C-3	70.5	70.5	77.9	77.7	82.5	82.5		
	(69.9)	(69.8)	(77.5)	(77.1)	(82.3)	(82.3)	(70.7)	(70.7)
C-4	69.9	69.9	72.2	75.1	76.8	76.8		
	(69.1)	(69.1)	(75.0)	(74.8)	(76.4)	(76.4)	(71.5)	(71.5)
C-5	69.6	69.6	81.6	81.6	83.8	83.8		
	(68.2)	(68.2)	(82.3)	(82.3)	(82.4)	(82.4)		
C-6	64.2	64.4	63.2	63.2				
	(63.8)	(63.8)	(62.8)	(62.8)	(61.7)	(61.7)		
n-C_4H_9	53.5	51.5	53.5	51.5				
	30.7	28.5	30.7	28.5				
	20.0	20.3	20.0	20.3				
	13.9	14.1	13.9	14.1				

[a] At 200 MHz (^1H) and at 50.2 MHz (^{13}C). [b] In brackets, in $(CD_3)_2SO$.

TABLE III
NMR data[a] for a solution of **11** in D_2O (δ in ppm J in Hz)

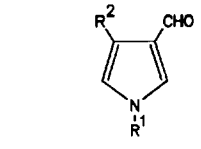
Atom	*β-p-EEE*	*β-f-EEE*	*α-f-EEE*
H-1′	7.14s	7.16s	–
H-3′	8.43s	8.28s	8.45s
CH_3	1.50s	1.78s	1.41s
Sugar moiety protons	←	3.2–4.0m	→
C-1′	165.5	165.2	–
C-2′	110.6	110.8	–
C-3′	191.2	192.2	191.5
CH_3	6.6	–	–
C-1	54.7	53.4	53.2
C-2	98.4	101.7	104.6
C-3	70.4	77.2	82.1
C-4	69.9	75.1	76.4
C-5	69.5	81.5	83.2
C-6	64.4	63.1	61.6

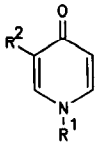
[a] At 200 MHz (^1H) and at 50.2 MHz (^{13}C).

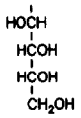
acetate of **7**. Compound **14** had UV, IR, and fluorescent spectra (see Experimental) that were similar to those of **12** and **13**. The NMR spectra indicated that, in solution in D_2O, **14** existed as an equilibrium mixture of the *β-p*, *β-f*, and *α-f* forms, with the first preponderating. The chiral substituent at position 1′ of the heterocycle makes the pairs of nuclei C-2′,6′ and C-3′,5′ magnetically non-equivalent, and, as in similar sugar derivatives[1], this was reflected in the doubling of signals observed in the ^{13}C-NMR spectrum. Compounds **12–14** were formed even when carefully purified preparations of NaMDA were used. Thus, the transformation of NaMDA into **6** in the reaction medium competes with its reaction with the aminoketoses. Furthermore, the formation of **12** and **13** implies that the aminoketoses **7** and **8** are partly cleaved under the reaction conditions, giving rise to ammonia and butylamine, respectively. The cleavage of *N*-substituted 1-amino-1-deoxyketoses to amines and osones occurs in the early stages of the Maillard reaction[2]. Compound **12** does not arise from the cleavage of **14**, since **14** is stable in aqueous solution at room temperature in the pH range 1–7.

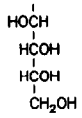
When an aqueous solution of the enaminal **9** (prepared by storing equimolecular amounts of the acetate of **7** and NaMDA in water at room temperature for 24 h) was heated, a deep brown colour and a biscuit-like aroma developed, and a mixture containing the pyrroles **15** and **16** and the pyridones **19** and **20** resulted. The reaction was faster at pH 9 when the transformation of **9** was complete in 1 h. The main products, **15** and **19**, isolated by TLC, were unstable, darkened rapidly, and could be obtained pure only in poor yields.

The cyclisation, with concomitant polymerisation, of the enaminal **10** occurred readily at room temperature and neutral pH. Heating an aqueous solution of this



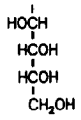


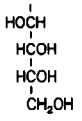
15 R^1 = H, R^2 = 

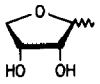
19 R^1 = H, R^2 = 

16 R^1 = H, R^2 = CH_2OH

20 R^1 = H, R^2 = CH_2OH

17 R^1 = n-C_4H_9, R^2 = 

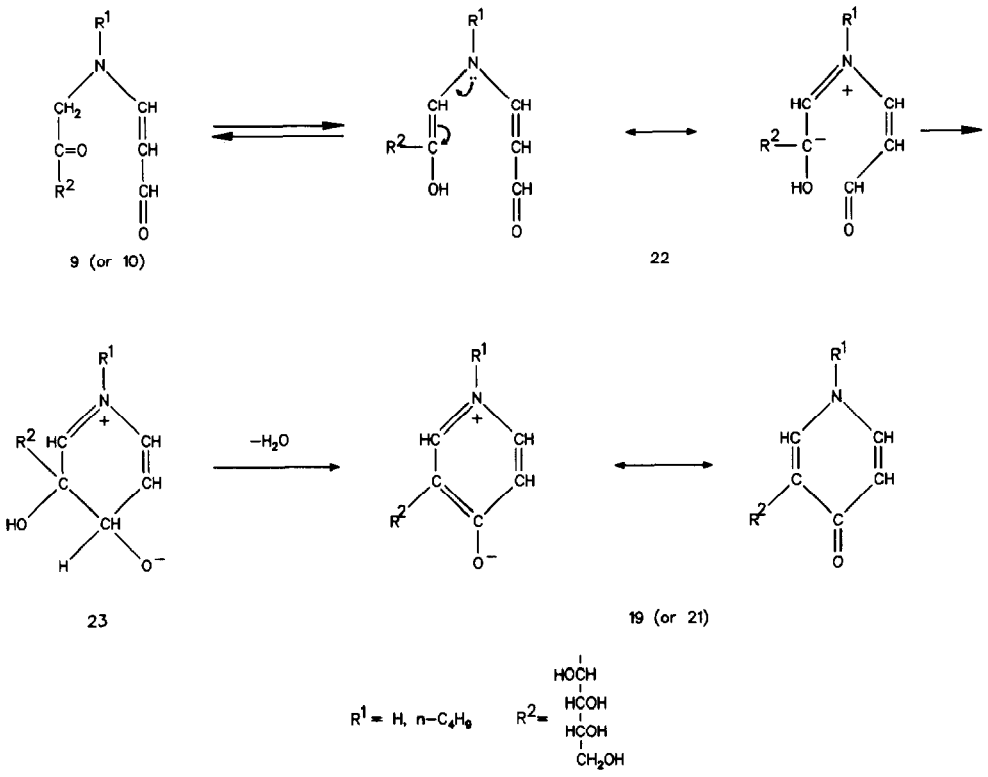
21 R^1 = n-C_4H_9, R^2 = 

18 R^1 = n-C_4H_9, R^2 = 

compound (or a solution of the oxalate of **8** and NaMDA after storage at room temperature until TLC indicated the complete transformation of **8** into **10**) for 1 h gave 35% of an insoluble, dark-brown material. The supernatant solution, which had a pear-like aroma, contained the pyrroles **17** and **18**, and the pyridone **21**. Under these conditions, a substantial amount (~ 25% by ^{1}H-NMR spectroscopy; 7% by recovery) of **10** survived. Prolongation of the reaction until **10** had reacted completely increased the yield of the polymer at the expense of the heterocyclic compounds. The pyridone **21** partly crystallised from the reaction medium, and **17**, **18**, and the remainder of **21** were obtained pure in low yields after repeated chromatography. These pyrroles were also unstable.

The structures of **15–21** were established on the basis of their analytical data and spectral properties. The anomeric configurations of **18α** and **18β** were assigned on the basis of their $[\alpha]_D$ values, the β configuration being attributed to the most levorotatory isomer[19]. This assignment was confirmed by the ^{1}H-NMR spectra which showed a smaller δ value and a larger $J_{1',2'}$ value for the α anomer in accordance with data in the literature[20].

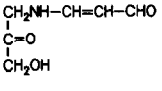
The formation of (alditol-1-yl)pyrroles from MDA and the aminoketoses **7** and **8**, through the intermediacy of the enaminals **9** and **10**, follows the well-established pattern of reaction of 1,3-dicarbonyl compounds with amino sugars[21,22]. The dehydration of such pyrroles as **17**, to yield (α,β-D-furanosyl)pyrroles **18α,β**, is also well documented[22]. A possible mechanistic pathway (Scheme 2) for the formation of the pyridones **19** and **21** involves enolisation of the *o* form of the



Scheme 2. Proposed mechanism for the formation of the 3-(D-*arabino*-tetritol-1-yl)-4-pyridones **19** and **21**.

enaminal (**9** or **10**) to the enaminol **22**, a mesomeric system that has an accumulation of negative charge on the carbon atom bearing the enol hydroxyl. Nucleophilic attack of this carbon on the aldehyde C=O gives the hydroxydihydropyridine **23**, dehydration of which yields the mesomeric 4-pyridone system (**19** or **21**).

The formation of 4-hydroxymethyl-3-pyrrolecarbaldehyde (**16**) and 3-hydroxymethyl-4-pyridone (**20**) implies the fission, at some stage, of the sugar chain. This scission does not occur in **15** and **19** since they were not transformed into **16** and **20**, respectively, when heated in neutral or weakly alkaline aqueous solution. The fission probably takes place by a retroaldol reaction of **9** to yield the enaminal (**24**) of 1-amino-3-hydroxypropanone, the dual cyclisation of which would produce **16** and **20**.



24

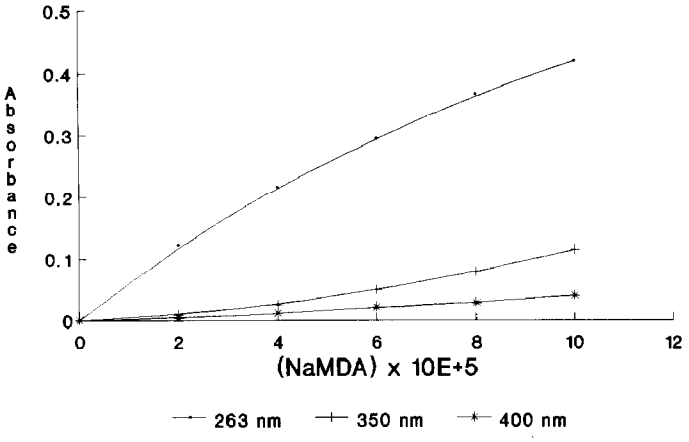


Fig. 1. Browning reaction of 1-butylamino-1-deoxy-D-fructose in the presence of MDA at different concentrations.

In the above experiments, the [MDA]:[amino ketose] ratios were much higher than those which may be present in food and in physiological media. In order to ascertain the influence that small concentrations of MDA may have in the browning reaction, 10^{-4} M solutions of **8**-oxalate containing 0–10^{-4} M NaMDA were heated at 100° and pH 3.5–4 for 1 h. No browning was observed in the absence of MDA and the solutions containing MDA yielded a dark, insoluble solid and developed a fruity aroma and a brown colour (λ_{max} 263, 350, and 400 nm), the intensity of which increased with increasing [MDA] (Fig. 1). TLC revealed the pyrroles **17** and **18** and the pyridone **21**, which are responsible for the absorption at 263 nm. The absorptions at 350 and 400 nm are attributed to the products of their polymerisation. In similar experiments performed at pH 7 [i.e., a pH value at which MDA (which has pK_a 4.46)[23] is almost completely dissociated], no absorption other than that due to NaMDA was observed, which indicated the inability of the MDA anion to react with the aminoketose.

The above results support the hypothesis that the MDA present in food and in biological fluids contributes to the browning and to the formation of flavour in the Maillard reaction. The avidity of MDA for Amadori compounds similar to **8** yields unstable 3-(1-deoxyketos-1-ylamino)acroleins, which are readily degraded to heterocycles structurally related to some intermediates[2] of the Maillard reaction, and finally to melanoidin-like polymers. Further work using oxidised food and Amadori compounds is in progress.

EXPERIMENTAL

General. — Unless stated otherwise, the methods used were as described[1]. TLC was performed on Silica Gel 60 F_{254} (Merck). Identification of compounds was based on comparisons of chromatographic and spectral properties.

Reaction of 1-amino-1-deoxy-D-fructose (**7**) *with malondialdehyde (MDA).* — To a solution of the acetate of **7** (0.478 g, 2 mmol) in water (10 mL) was added NaMDA monohydrate (0.224 g, 2 mmol), and the solution was stored at room temperature for 24 h. TLC (ethyl acetate–methanol–acetic acid–water, 6:2:1:1) then revealed **9** (R_F 0.46, main component), **12** (R_F 0.70), **14** (R_F 0.62), and traces of an unidentified compound (R_F 0.07). Evaporation of the solvent and preparative TLC (ethyl acetate–methanol–water, 4:1:1) of the residue afforded **9** (0.280 g, 60%), **12** (3 mg, 1%), and **14** (31 mg, 5%).

1-Deoxy-1-(3-oxo-1-propenylamino)-D-fructose (**9**) was amorphous and had $[\alpha]_D^{15}$ −61° (*c* 0.49, water); $\lambda_{max}^{H_2O}$ 282 nm (ϵ 29380); ν_{max}^{KBr} 3335 (OH, NH), 1650 (C=O), 1601 and 1555sh cm^{-1} (C=C–NH). The NMR data appear in Table I.

Anal. Calcd for $C_9H_{15}NO_6$: C, 46.35; H, 6.48; N, 6.01. Found: C, 46.31; H, 6.53; N, 5.87.

4-Methyl-1,4-dihydro-3,5-pyridinedicarbaldehyde (**12**) had mp 169–171° (from ethanol–water) and was identical with an authentic sample prepared from the sodium salt of **6** and ammonium acetate[9].

1-Deoxy-1-(3,5-diformyl-4-methyl-1,4-dihydropyridin-1-yl)-D-fructose (**14**) was also obtained by treating the sodium salt of **6** (0.475 g, 2 mmol) with the acetate of **7** (0.478 g, 2 mmol) in water (10 mL) for 24 h. Concentration of the mixture and preparative TLC (ethyl acetate–methanol, 5:1) of the residue yielded **14** (0.188 g, 30%), mp 110–111° (from ethanol); $[\alpha]_D^{15}$ −30° (*c* 0.83, water); λ_{max}^{MeOH} 235, 262, and 384 nm (ϵ 10080, 8440, and 8880); λ_{max}^{Ex} 391 nm, λ_{max}^{Em} 453 (RMI, 0.92); ν_{max}^{KBr} 3400 (OH), 1666 (C=O), and 1570 cm^{-1} (C=C). Mass spectrum: *m/z* 313 (1%, M^+), 151 (11), 136 (100), 108 (10), 80 (40), and 53 (20). NMR data: ^1H, δ 0.95 (d, 3 H, *J* 6.4 Hz, Me-4′), 3.4–5.1 (m, 7 H, H-1,1,3,4,5,6,6), 3.59 (m, 1 H, H-4′), 7.14 (s, 2 H, H-2′,6′), and 9.03 (s, 2 H, 2 CHO); ^{13}C, *β-p* form, δ 60.2 (C-1), 98.4 (C-2), 70.5 (C-3), 69.8 (C-4), 69.4 (C-5), 64.5 (C-6), 22.2 (Me-4′ or C-4′), 22.7 (C-4′ or Me-4′), 123.40 (C-3′ or C-5′), 123.5 (C-5′ or C-3′), 152.36 (C-2′ or C-6′), 152.44 (C-6′ or C-2′), and 193.7 (CHO); *β-f* form, δ 59.3 (C-1), 101.4 (C-2), 77.5 (C-3), 74.8 (C-4), 81.6 (C-5), 63.0 (C-6), 22.4 (Me-4′ and C-4′), 123.6 (C-3′,5′), 151.9 (C-2′ or C-6′), 152 (C-6′ or C-2′), and 193.7 (CHO); *α-f* form, δ 58.8 (C-1), 104.8 (C-2), 82.7 (C-3), 77.1 (C-4), 82.8 (C-5), 62.0 (C-6), 22.5 (Me-4′ and C-4′), 123.2 (C-3′,5′), 152.7 (C-2′ or C-6′), 152.9 (C-6′ or C-2′), and 193.7 (CHO).

Anal. Calcd for $C_{14}H_{19}NO_7$: C, 53.67; H, 6.11; N, 4.47. Found: C, 53.38; H, 6.06; N, 4.30.

1-Deoxy-1-(2-methyl-3-oxo-1-propenylamino)-D-fructose (**11**). — To a solution of the acetate of **7** (0.478 g, 2 mmol) in water (10 mL), was added sodium methylmalondialdehyde (0.216 g, 2 mmol), and the solution was heated at 90° for 1.5 h. Concentration and preparative TLC (ethyl acetate–methanol–triethylamine–water, 6:2:1:1) of the residue afforded **11** (0.345 g, 70%), isolated as a hygroscopic foam, $[\alpha]_D^{15}$ −31° (*c* 0.58, water); $\lambda_{max}^{H_2O}$ 290 nm (ϵ 31680); ν_{max}^{KB} 3380 (OH, NH), 1653 (C=O), and 1589 cm^{-1} (C=CN–H). The NMR data appear in Table III.

Anal. Calcd for $C_{10}H_{17}NO_6 \cdot 0.5H_2O$: C, 46.86; H, 7.08; N, 5.47. Found: C, 46.70; H, 7.07; N, 5.18.

Reaction of 1-butylamino-1-deoxy-D-fructose (**8**) *with NaMDA.* — A solution of the oxalate of **8** (1.63 g, 5 mmol) and NaMDA monohydrate (1.12 g, 10 mmol) in water (10 mL) was stored at room temperature overnight, then concentrated, and the residual syrup was treated with methanol (10 mL). The solid was removed and the filtrate was concentrated. Preparative TLC (chloroform–methanol, 5:2) of the residue afforded **10** (0.854 g, 60%) and **13** (8 mg, 1%).

1-[*N*-Butyl-*N*-(3-oxo-1-propenyl)amino]-1-deoxy-D-fructose (**10**) was a yellow foam, $[\alpha]_D^{15}$ −42° (*c* 0.45, water); $\lambda_{max}^{H_2O}$ 291 nm (ϵ 43980); ν_{max}^{KBr} 3347 (OH, NH), 1650sh (C=O), and 1601 cm^{-1} (C=C). The NMR data appear in Table II.

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.97; H, 8.01; N, 4.89. Found: C, 53.83; H, 8.02; N, 4.64.

1-Butyl-4-methyl-1,4-dihydro-3,5-pyridinedicarbaldehyde (**13**) had mp 116–117° (from methanol), and was identical with an authentic specimen prepared from **6** and butylamine[9].

Cyclisation of the enaminal **9**. — A solution of the acetate of **7** (1.434 g, 6 mmol) and NaMDA monohydrate (0.672 g, 6 mmol) in water (45 mL) was stored at room temperature until the transformation of the aminoketose into **9** was complete. The pH was adjusted to 9 with satd aq Na_2CO_3 and the solution was heated at 90° for 1.5 h. TLC (ethyl acetate–methanol–acetic acid–water, 6:2:1:1) then indicated the absence of **9** and the formation of **15** (R_F 0.36), **16** (R_F 0.51), **19** (R_F 0.53), and **20** (R_F 0.82). The solution was extracted with ethyl acetate (4 × 25 mL), the combined extracts were dried (Na_2SO_4), and the solvent was evaporated. Preparative TLC of the syrupy residue (130 mg) afforded **16** (10 mg, < 1%) and **20** (5 mg, < 1%).

The aqueous fraction was concentrated to a syryp that was extracted with hot ethanol, and the extract was concentrated. Preparative TLC (ethyl acetate–methanol–water–triethylamine) of the syrupy residue afforded **15** (35 mg, 2%) and **19** (20 mg, 1%).

4-(D-*arabino*-Tetritol-1-yl)-3-pyrrolecarbaldehyde (**15**) was a syrup that darkened in the air and had $[\alpha]_D^{15}$ −35° (*c* 0.8, water); $\lambda_{max}^{H_2O}$ 253 and 290sh nm (ϵ 9800 and 4835). NMR data (D_2O): ^1H, δ 3.40–3.70 (m, 4 H, H-2′,3′,4′a,4′b), 5.05 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 6.85 (dd, 1 H, $J_{2,5}$ 2.0, $J_{5,CHO}$ 0.8 Hz, H-5), 7.58 (d, 1 H, H-2), and 9.44 (d, 1 H, CHO). This compound was unstable and turned red in the air.

Anal. Calcd for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.51. Found: C, 49.87; H, 6.28; N, 6.19.

4-Hydroxymethyl-3-pyrrolecarbaldehyde (**16**) had mp 93–95° (from ethanol–water); $\lambda_{max}^{H_2O}$ 252 and 290sh nm (ϵ 10730 and 4850); ν_{max}^{KBr} 3294 (OH, NH), 2861 and 2777 (CH, aldehyde), 1641 (C=O), 1563 and 1517 cm^{-1} (C=C pyrrole). NMR data (D_2O): ^1H, δ 4.56 (s, 2 H, CH_2OH), 6.78 (dd, 1 H, $J_{2,5}$ 1.9, $J_{5,CHO}$ 0.7 Hz, H-5), 7.52 (d, 1 H, H-2), and 9.48 (d, 1 H, CHO). Mass spectra: *m/z* 125 (M^+, 94%), 107 (90), 94 (25), and 78 (100); *m/z* 125.048 (calc. for $C_6H_7NO_2$: 125.123).

3-(D-*arabino*-Tetritol-1-yl)-4-pyridone (**19**) had mp 190–191° (dec, from water), $[\alpha]_D^{15}$ −4.8° (*c* 0.42, Me_2SO); $\lambda_{max}^{H_2O}$ 255sh and 296 nm (ϵ 3450 and 16610); ν_{max}^{KBr}

3410 (NH), 3210 (OH), 1665 (C=O), and 1545 cm^{-1} (C=C). NMR data (D_2O): ^1H, δ 3.45–3.90 (m, 4 H, H-2′,3′,4′a,4′b), 5.07 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1′), 6.25 (dd, 1 H, $J_{2,5}$ 2.4, $J_{5,6}$ 3.8 Hz, H-5), 7.06 (dd, 1 H, $J_{2,6}$ 1.3 Hz, H-6), and 7.14 (dd, 1 H, H-2). Mass spectrum: m/z 215 (M^+, 2%), 125 (30), 94 (100), 67 (25), and 60 (30). This compound was unstable and turned grey in the air.

Anal. Calcd for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.00; H, 6.04; N, 6.51.

3-Hydroxymethyl-4-pyridone (**20**) had mp 105–107° (from ethanol–water); $\lambda_{max}^{H_2O}$ 250sh and 291 nm (ϵ 5500 and 19420), ν_{max}^{KBr} 3425, 3372, and 3321 (NH, OH), 1655 (C=O), and 1551 cm^{-1} (C=C). NMR data (MeOD): ^1H, δ 4.56 (s, 2 H, CH_2OH), 6.33 (dd, 1 H, $J_{2,5}$ 2.5, $J_{5,6}$ 3.9 Hz, H-5), 7.10 (dd, 1 H, $J_{2,6}$, 1.3 Hz, H-6), and 7.18 (dd, 1 H, H-2). Mass spectra: m/z 125 (M^+, 23), 94 (100), and 66 (44); m/z 125.0479 (calc. for $C_6H_7NO_2$: 125.123).

Cyclisation of the enaminal **10**. — (*a*) A solution of the oxalate of **8** (1.620 g, 5 mmol) and NaMDA monohydrate (1.120 g, 10 mmol) in water (25 mL) was stored at room temperature until the reaction was complete (TLC, UV). The solution, with pH 4, was heated for 1 h during which time a black, amorphous solid precipitated and a pear-like aroma developed. The solid was collected, washed with water, and dried. This material (0.151 g) contained (TLC; chloroform–methanol, 1:1) small proportions of the 4-pyridone **21** (R_F 0.72) and a black product of R_F 0.0. Extraction with hot ethanol afforded **21** (15 mg).

The filtrate and the washings were combined and concentrated, and the residue was treated with methanol. The precipitated solid (sodium hydrogen oxalate) was removed, and the filtrate was concentrated to leave a syrup that contained (TLC; chloroform–methanol, 10:1) **10** (R_F 0.15), **17** (R_F 0.32), **18α** (R_F 0.58), **18β** (R_F 0.63), and **21** (R_F 0.37). Column chromatography (chloroform–methanol, 10:1) afforded fractions enriched in each of the above products and preparative TLC then afforded **17** (0.20 g, 15%), **18α** (25 mg, 2%), **18β** (27 mg, 2%), and **21** (68 mg, 5%).

1-Butyl-4-(D-*arabino*-tetritol-1-yl)-3-pyrrolecarbaldehyde (**17**) had mp 125–126° (from ethanol), $[\alpha]_D^{15}$ −40° (*c* 0.45, methanol); λ_{max}^{EtOH} 260 nm (ϵ 12620); ν_{max}^{KBr} 3277 (OH), 2816 and 2727 (CH, aldehyde), 1653 (C=O), 1549 and 1526 cm^{-1} (C=C, pyrrole). NMR data (MeOD): ^1H, δ 0.95 (t, 3 H, *J* 7.3 Hz, Me), 1.30, 1.78, and 3.97 [2 m and t, each 2 H, *J* 7.1 Hz, $(CH_2)_3$], 3.5–3.9 (m, 4 H, H-2′,3′,4′a,4′b), 5.16 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-1′), 6.90 (d, 1 H, $J_{2,5}$ 2.2 Hz, H-5), 7.58 (d, 1 H, H-2) and 9.58 (s, 1 H, CHO); ^{13}C, δ 14.4, 20.2, 33.4 and 50.2 (Bu), 64.1 (C-4′), 67.21 (C-3′), 72.7 (C-2′), 75.6 (C-1′), 123.5 (C-5), 123.9 (C-3 or C-4), 126.9 (C-4 or C-3), 134.4 (C-2), and 188.1 (CHO). Mass spectrum: m/z 271 (M^+, 1%), 253 (3), 235 (4), 180 (100) 150 (8), 124 (12), and 94 (5).

Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.81; H, 7.81; N, 5.02.

1-Butyl-4-(α-D-erythrofuranosyl)-3-pyrrolecarbaldehyde (**18α**) was a syrup that darkened rapidly and had $[\alpha]_D^{15}$ −37° (*c* 0.35, methanol); λ_{max}^{MeOH} 258 nm (ϵ 7680);

ν_{max}^{KBr} 3360 (OH), 1657 (C=O), 1559 and 1526 cm^{-1} (C=C, pyrrole). NMR data (MeOD): ^1H, δ 0.94 (t, 3 H, J 7.3 Hz, Me), 1.31, 1.73, and 3.95 [2 m and t, each 2 H, J 7.1 Hz, $(CH_2)_3$], 3.80 (m, 1 H, H-4'a), 4.14 (dd, 1 H, $J_{1',2'}$ 6.6, $J_{2',3'}$ 4.4 Hz, H-2'), 4.25 (m, 2 H, H-3',4'b), 4.98 (dd, 1 H, $J_{5,1'}$ 0.8 Hz, H-1'), 6.87 (dt, 1 H, $J_{2,5}$ 2.3, $J_{5,CHO}$ 0.8 Hz, H-5), 7.56 (d, 1 H, H-2), and 9.65 (d, 1 H, CHO); ^{13}C, δ 13.9, 20.7, 34.1, and 50.8 (Bu), 73.6 (C-4'), 72.6, 78.2, and 79.9 (C-1',2',3'), 123.9 (C-5), 124.6 (C-3 or C-4), 125.0 (C-4 or C-3), 134.7 (C-2), and 187.5 (CHO).

Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64: H, 7.56; N, 5.53. Found: C, 61.27; H, 7.75; N, 5.31.

1-Butyl-4-(β-D-erythrofuranosyl)-3-pyrrolecarbaldehyde (**18β**) had mp 134–136° (from methanol), $[\alpha]_D^{15}$ −96.5° (*c* 0.43, methanol); λ_{max}^{MeOH} 258 (ϵ 11910); ν_{max}^{KBr} 3449 (OH), 2836 and 2729 (CH, aldehyde), 1655 (C=O), 1550 and 1524 cm^{-1} (C=C, pyrrole). NMR data (MeOD): ^1H, δ 0.95 (t, 3 H, J 7.3 Hz, Me), 1.37, 1.77, and 3.95 [2 m and t, each 2 H, J 7.1 Hz, $(CH_2)_3$], 3.76 (dd, 1 H, $J_{3',4'a}$ 7.1, $J_{4'a,4'b}$ 8.4 Hz, H-4'a), 3.96 (dd, 1 H, $J_{3',4'b}$ 7.1 Hz, H-4'b), 4.24 (dd, 1 H, $J_{2',3'}$ 4.9, $J_{1',2'}$ 3.4 Hz, H-2'), 4.48 (dt, 1 H, H-3'), 5.25 (dd, 1 H, $J_{5,1'}$ 0.8 Hz, H-1'), 6.86 (dt, 1 H, $J_{5,CHO}$ 0.8, $J_{2,5}$ 2.3 Hz, H-5), 7.50 (d, 1 H, H-2), and 9.61 (d, 1 H, CHO).

Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.62; H, 7.25; N, 5.66.

1-Butyl-3-(D-*arabino*-tetritol-1'-yl)-4-pyridone (**21**) had mp 180–182° (from ethanol), $[\alpha]_D^{15}$ 0° (*c* 0.5, Me_2SO); λ_{max}^{EtOH} 290 and 255sh nm (ϵ 21460 and 7570); ν_{max}^{KBr} 3451, 3354 and 3285 (OH), 1653 (C=O), and 1520 cm^{-1} (C=C). Mass spectra: *m/z* 271 (M^+, 3%), 150 (100), 122 (10), 94 (35), and 66 (10); *m/z* 271.1464 (calc. for $C_{13}H_{21}NO_5$: 271.319). NMR data [$(CD_3)_2SO$]: ^1H, δ 0.85 (t, 3 H, J 7.3 Hz, Me), 1.22, 1.61, and 4.26 [3 m, each 2 H, $(CH_2)_3$], 3.4–3.8 (m, 4 H, H-2',3',4'a,4'b), 5.02 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1'), 6.14 (dd, 1 H, $J_{2,5}$ 2.3, $J_{5,6}$ 4.1 Hz, H-5), 7.01 (dd, 1 H, $J_{2,6}$ 1.8 Hz, H-6), and 7.20 (dd, 1 H, H-2); ^{13}C, δ 13.8, 19.4, 33.3 and 48.7 (Bu), 63.4 (C-4'), 71.4, 72.7, and 74.6 (C-1',2',3'), 108.2 (C-5), 119.8 (C-3), 126.8 (C-2 or C-6), 131.2 (C-6 or C-2), and 190.5 (C-4).

Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.27; H, 8.02; N, 4.83.

(*b*) A solution of **10** (50 mg) in D_2O (0.5 mL) was heated at 90°. Monitoring (TLC, ^1H-NMR spectroscopy) revealed, after 1 h, **10**, **17** + **18α** + **18β**, and **21** in the ratios 5 : 10 : 6.

Browning reaction of **8** *in the presence of MDA.* — Aliquots of a 10^{-4} M solution of **8**-oxalate containing NaMDA (10^{-5}–10^{-4} M, pH 3.5–4.0) were heated at 100° for 1 h and then cooled. The black solid was removed and the absorptions of the fruity-smelling, dark-coloured supernatant solutions were measured in the 200–500 nm range against a blank of 10^{-4} M **8**-oxalate. The intensities of the absorptions with λ_{max} at 263, 350, and 400 nm increased with increasing [NaMDA] (Fig. 1). TLC of these solutions revealed **17**, **18α,β**, and **21**.

Similar experiments in a phosphate buffer of pH 7 only showed λ_{max} 266 nm, the intensity of which varied linearly with [NaMDA].

ACKNOWLEDGMENTS

We thank the "Comisión Interministerial de Ciencia y Tecnología" (grant ALI88-0169), the C.S.I.C., and the Junta de Andalucia for financial support.

REFERENCES

1 A. Gómez-Sánchez, I. Maya, and I. Hermosín, *Carbohydr. Res.*, 200 (1990) 167–180.
2 (a) J.E. Hodge, *J. Agric. Food Chem.*, 1 (1953) 928–943; (b) *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, and R. Liardon (Eds.), Birkhäuser Verlag, Basel, 1990.
3 L.J. Farmer and D.S. Mottram, *J. Sci. Food Agric.*, 53 (1990) 505–525, and references therein.
4 M. Toyomizu, *Nippon Suisan Gakkaishi*, 36 (1970) 847–849, 871; J. Pokorny, in C. Eriksson (Ed.), *Maillard Reactions in Food: Chemical, Physiological, and Technological Aspects*, Pergamon, Oxford, 1981, pp. 421–428.
5 W.A. Pryor (Ed.), *Free Radicals in Biology*, Vols. 1 and 2, Academic Press, New York, 1976.
6 K.S. Chio and A.L. Tappel, *Biochemistry*, 8 (1969) 2821–2827, 2827–2832.
7 K. Vidysagar, S.S. Arya, K.S. Premavalli, D.B. Parihar, and H. Nath, *J. Food Sci. Technol.*, 11 (1974) 73–75.
8 K. Kikugawa and Y. Ido, *Lipids*, 19 (1984) 600–608.
9 A. Gómez-Sánchez, I. Hermosín, and I. Maya, *Tetrahedron Lett.* 31 (1990) 4077–4080.
10 J. Dabrowski and K. Kamienska-Trela, *J. Am. Chem. Soc.*, 98 (1976) 2826–2834.
11 J. Terpinski and J. Dabrowski, *J. Mol. Struct.*, 4 (1969) 285–291.
12 H. Roper, S. Roper, K. Heyns, and B. Meyer, *Carbohydr. Res.*, 116 (1983) 183–195.
13 W. Funcke and A. Klemer, *Justus Liebigs Ann. Chem.*, (1975) 1232–1235.
14 W. Funcke and A. Klemer, *Carbohydr. Res.*, 50 (1976) 9–13.
15 W. Funcke, C. von Sonntag, and C. Triantaphylides, *Carbohydr. Res.*, 75 (1979) 305–309.
16 A. Gómez-Sánchez, M.G. García Martín, and C. Pascual, *Carbohydr. Res.*, 149 (1986) 329–345.
17 D.D. Tavernier and M.J.O. Antennis, *J. Magn. Reson.*, 11 (1978) 628–631.
18 K. Kikugawa, T. Nakahara, and S. Sakurai, *Chem. Pharm. Bull.*, 35 (1987) 4656–4660.
19 A. Gómez-Sánchez and A. Rodríguez Roldán, *Carbohydr. Res.*, 22 (1972) 53–62; J. Fernández-Bolaños, J. Fuentes Mota, J. Barragán Pérez, and M.A. Pradera de Fuentes, *An. Quim.*, 74 (1978) 336–338.
20 J. Fernández-Bolaños, J. Fuentes Mota, and I. Robina, *An. Quím.*, 80 (1984) 123–126.
21 F. García González and A. Gómez-Sánchez, *Adv. Carbohydr. Chem.*, 20 (1963) 303–347.
22 A. Gómez-Sánchez, E. Toledano, and M. Gómez Guillén, *J. Chem. Soc., Perkin Trans. 1*, (1974) 1237–1243, and references therein.
23 M.M. Osman, *Helv. Chim. Acta*, 55 (1972) 239–244.