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# CLEAVAGE AND OLIGOMERIZATION OF MALONDIALDEHYDE UNDER PHYSIOLOGICAL CONDITIONS

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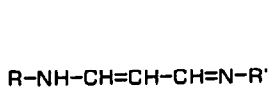
**Summary:** Malondialdehyde slowly oligomerizes in aqueous solution at pH 5-7 and room temperature. Cleavage of the dialdehyde also takes place under these conditions yielding ethanal, which further reacts with the excess of malondialdehyde to yield 2,4-dihydroxymethylene-3-methylglutaraldehyde.

Malondialdehyde (MDA) is a naturally occurring compound produced in substantial quantity in mammalian tissues and in lipid-rich foods as an end product of polyunsaturated lipid peroxidation. MDA has been associated with various biological processes such as cross-linking of biological macromolecules,[1] cell aging,[2] and the deterioration of food.[3] The estimation of MDA in oxidized lipids or tissues by the 2-thiobarbituric acid (TBA) method[4] is often used as an index of lipid peroxidation and rancidity. However, the TBA test is nonspecific for free MDA, and some of its precursors and/or derivatives have been shown to give a positive test.[5] Tappel et al.[2] considered that the fluorescent lipofuscin pigments accumulated in aging organisms as a result of in vivo lipid peroxidation are the 1-amino-3-iminopropenes (**1**) derived from MDA and bioamines. On the other hand, Kikugawa et al.[6] found that MDA reacts with amines and amino acids under physiological conditions to yield fluorescent N-substituted 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehydes (e.g., **3**), which suggested that structures of this type are also present in the lipofuscin pigments. We report here on some cleavage and oligomerization reactions that MDA undergoes under physiological conditions, and on their chemical and biological implications.

NaMDA (**6**) monohydrate, m.p. 240-242º (dec.), was prepared (75% after recrystallization from acetone-water) by hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) catalyzed by Dowex 50W-X8, and subsequent neutralization with M NaOH to pH 7.[7] Column chromatography, followed by preparative t.l.c., of a concentrate of the mother liquors of **6** afforded the mono-sodium salts of 2,4-dihydroxymethylene-3-(2,2-dimethoxyethyl)glutaraldehyde monohydrate (**7**), m.p. 139-140º (dec.), (6%), 2,4-dihydroxymethylene-3-methylglutaraldehyde dihydrate (**8**), m.p. 250-252º (dec.), (ca. 1%), and (E)-(3'-oxo-1'-propenyl)malondialdehyde (**13**), m. p. 245-247º (dec.), (ca. 0.5%).[8] The structure of **7** suggests that it arises from the condensation of **6** with 3,3-dimethoxypropanal, a known[9] side product of the hydrolysis of TMP. Similarly, compound **8** might be formed by condensation of **6** with ethanal, and indeed we found that **8** can be readily obtained (60% after recrystallization from methanol) by reaction of one mole of ethanal with two moles of **6** in aqueous solution (pH 7-8) at room temperature. The similar reactions with methanal, butanal, and hexanal afforded compounds **9** (10%), m.p. 275º (dec., from methanol), **10** (74%) , m.p. 255º (dec., from methanol), and **11** (78%), m.p. 270º (dec., from



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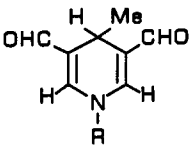
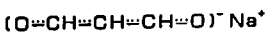
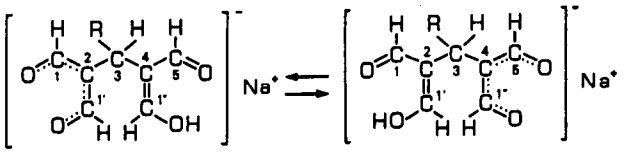


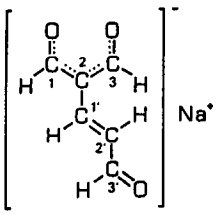
2 R = H
3 R = Me
4 R = n-$C_4H_9$
5 R = cyclo-$C_6H_{11}$



6



A B



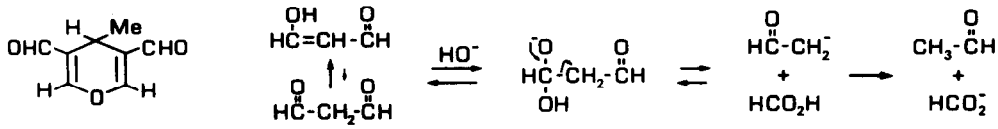
13

7 R = $CH_2CH(OMe)_2$
8 R = Me
9 R = H
10 R = n-$C_3H_7$
11 R = n-$C_6H_{11}$
12 R = $CH_2CHO$

methanol), respectively.

The structures of **7-11** followed from their analytical and spectral data. The NMR spectra showed that, in $D_2O$, these compounds have a symmetrical structure; this is considered to be due to the fact that the anions of **7-11** are acids in aqueous solution and a rapid prototropic equilibrium is established between the two equivalent forms **A** and **B**.[10] Compound **8** was acidified with an excess of 0.5M HCl, and the mixture was titrated with 0.1006M NaOH at 25° and a fixed ionic strength (I=0.1, KCl); the $pK_a$ values of 2,4-dihydroxymethylene-3-methylglutaraldehyde thus obtained were (with activity corrections) $pK_{a(1)}$=4.93 and $pK_{a(2)}$=8.00, the former being very near to that of MDA ($pK_a$=4.46).[11] Reactions of **8** with ammonia or the appropriate amine in water solution at room temperature yielded the 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehydes **2** (16%), m.p. 169-171° (from methanol; lit.,[12] 155-168°), **3** (55%), m.p. 145-148° (from methanol; lit.,[13] 144-147°), **4** (10%), m.p. 116-117° (from methanol), and **5** (35%), m.p. 151-152° (from methanol-water). Treatment of **8** with HCl in ether afforded 4-methylpyrane-3,5-dicarbaldehyde (**14**) (20%), m.p. 85-87° (from ether). The analytical and spectral properties of compound **13** indicated the "quasi-dimeric" structure, and the position and the E-configuration of the carbon-carbon double bond was deduced from the presence in the $^1H$ NMR spectrum (in $D_2O$) of signals at δ 7.55 (d, $J_{1',2'}$ 15.4 Hz, 1H, H-1'), 6.97 (dd, $J_{1',2'}$ 15.5 Hz, $J_{2',3'}$ 8.7 Hz, 1 H, H-2'), and 9.26 (d, $J_{2',3'}$ 8.7 Hz, 1 H, H-3'), in addition to the two-proton singlet at δ 8.94 due to H-1 and H-3.

When a water solution of MDA,[14] or of NaMDA, was adjusted to pH 4.5-5.0 (i.e., ca. the $pK_a$ of MDA), and this pH value was maintained constant by periodical additions of Dowex 50W-X8 resin or M HCl, the "quasi-trimeric" form (**12**) of MDA was slowly formed (t.l.c.), in addition to smaller



14 15 Scheme

amounts of compounds **8** and **13**.[15] After 2 days at room temperature, the transformation of MDA was complete. Neutralization of the reaction mixture with M NaOH, followed by concentration and chromatography, afforded **12** (31%), m.p. 178-181° (dec.). This compound can be readily obtained (90%) by acid hydrolysis (pH 1) of its acetal **7**, followed by neutralization (M NaOH), concentration, and addition of acetone to the syrupy residue.

The formation of **8** from MDA implies that the latter compound is cleaved in aqueous solution at pH 5-7, yielding ethanal. In order to verify this, freshly prepared 0.05M solutions of MDA, contained in closed vials, were adjusted to various pH conditions (3-8) and incubated at 35°. After 15 min., the vials were connected to a gas-chromatograph, and the head-space analyzed.[16] Ethanal was detected in the pH range 4-7, the highest yield being observed at pH 5.5; under these conditions, ca. 9% of the head-space content was ethanal. This aldehyde was isolated (1%) as its 2,4-dinitrophenylhydrazone, m.p. and mixed m.p. 167-168° (dec.), from a solution of MDA kept at pH 5.5 and 35° for 15 min. The formation of ethanal can be viewed as a hydrolytic cleavage of the dicarbonyl form (**15**) of MDA (see the **Scheme**) similar to that undergone by 1,3-diketones under more strongly basic conditions. The liability of **15** to hydrolysis may be due to the greater electrophilicity of the aldehyde carbonyls of this compound relative to those of 1,3-diketones. The cleavage is restricted to the pH range 4-7 because at higher pH values, MDA exists almost exclusively as its anion, and at pH below 4 there is not enough concentration of hydroxyl anions.

The 2,4-dihydroxymethyleneglutaraldehydes **7-11** are most likely formed by addition of the MDA anion to the alkylidene-malondialdehydes resulting from the condensation of MDA with the added aldehydes, or with the ethanal present in the reaction medium. Likewise, the reaction of two molecules of MDA would yield (3'-oxopropylidene)-malondialdehyde which, by allylic migration of the carbon-carbon double bond, would afford **13**, and, by addition of another molecule of MDA, the trimeric structure **12**.

The above results throw some light on some unclear aspects of MDA chemistry. The first concerns the estimation of MDA by the TBA method. Several authors[5,17] have pointed out that the TBA test is given by a group of chemically related compounds, some of them considered[17] to be low-molecular-weight polymers of MDA, which are present in MDA preparations of different sources. The ready formation of compounds such as **8**, **12**, and **13**, and their reaction with the TBA reagent, suggest that they can accompany MDA in its preparations, and that they are some of these TBA-reactive substances. The ready formation of **8** from MDA alone, or of **8-11** from MDA and the appropriate aldehyde, and the subsequent transformation of these compounds into the 1,4-dihydropyridine-3,5-dicarbaldehydes **2-5** by treatment with $NH_3$ or amines, explain the observation[6] that **2-5** (and compounds of similar structure) are obtained by the one-pot reaction of MDA-aldehyde-amine (or amino

acid). Furthermore, 2,4-diacylglutaraldehydes similar to **7-11** may be intermediates in the Hantzsch 1,4-dihydropyridine synthesis[18] from 1,3-dicarbonyl compounds, aldehydes and $NH_3$. From the biological point of view, it should be noted that MDA coexists in physiological media with alkanals,[19] and therefore compounds similar to **11** may be formed in these media, in addition to the derivatives **8, 12,** and **13** arising from the cleavage and oligomerization of MDA. These tri- and tetra-aldehydes may act modifying biological macromolecules. Finally, the formation of the 3-alkyl-2,4-dihydroxymethyleneglutaraldehydes **8-12** adds support to Kikugawa's model[6] for the formation of fluorescent substances from MDA and proteins.

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10. For example, compound **8** exhibited signals at $\delta$ 8.24 (s, 4 H, H-1,1',1",5), 4.15 (q, 1 H, J 7.6 Hz, H-3), 1.25 (d, 3 H, J 7.6 Hz, Me) in its $^1H$ spectrum, and at $\delta$ 188.8 (C-1,1',1", 5), 126.6 (C-2, C-4), 20.0 (C-3), and 16.8 (Me) in its $^{13}C$ spectrum.
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14. Prepared immediately before use by hydrolysis of TMP (Dowex 50W-X8, 2 h, room temperature). The 0.05M solution of MDA has pH 3.4, and under these conditions MDA remains unchanged for ca. 2 h at room temperature.
15. Compounds **7-13** give a positive TBA test very similar to that of NaMDA ( $\lambda_{max}$ 530 nm, $\epsilon$ 144500). The $\lambda_{max}$ and $\epsilon$ values for each of the compounds are ( $\lambda_{max}$, in nm; $\epsilon$ in brackets): **7**, 530 (50500) and 626 (11000); **8**, 530 (205600); **9**, 530 (98000); **10**, 530 (168800); **11**, 530 (90900); **12**, 530 (49500) and 626 (9500); and **13**, 530 (23600) and 626 (10400). Compounds **6, 7, 8, 12,** and **13** can be separated by t.l.c. (silica gel; ethyl acetate-MeOH-$Et_3N$, 6:1:1; detection with UV light and TBA spray). The mobilities ($R_M$) referred to that of NaMDA ($R_M$=1) are: **7**, 3.75; **8**, 3.33; **12**, 1.25, and **13**, 1.50.
16. Gas chromatography was performed on a Perkin-Elmer Sigma 3B instrument fitted with a headspace adapter, using a Carbowax 20M (5% on Cromosorb G-AW, 80-100 mesh) column. GC conditions were: sample temperature, 60º; column temperature, 90º; detector temperature, 250º; carrier gas (nitrogen) flow rate, 20 mL/min. Under these conditions, the retention times of ethanal and of MDA were 1.58 min. and 2.70 min., respectively.
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