Dimer and Trimer of Malonaldehyde

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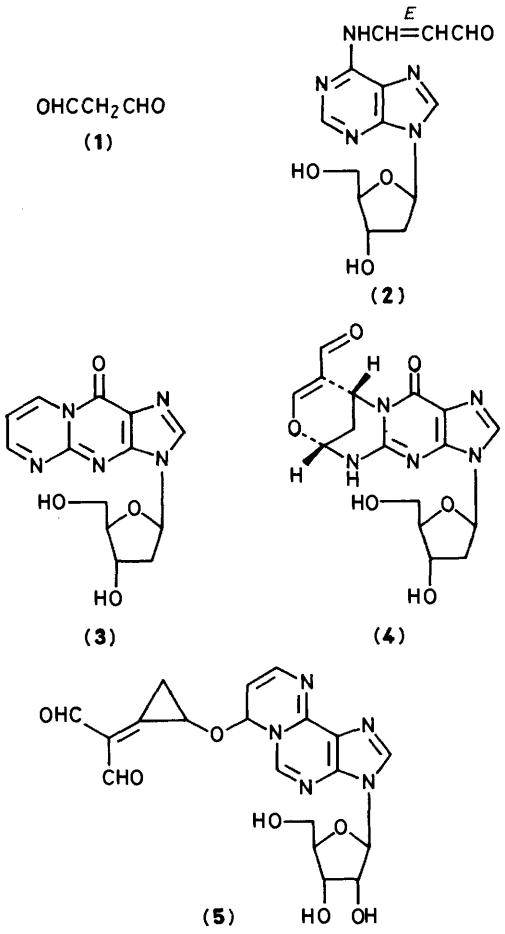
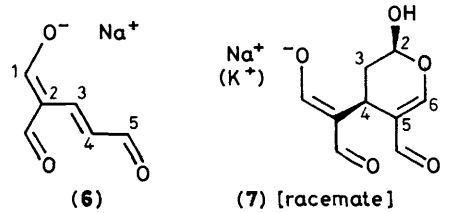
Self-condensation of malonaldehyde in water at pH 4.2 gives (*E*)-2-formylpent-3-ene-1,5-dial (**6**) and 4-(1,3-dioxopropan-2-yl)-3,4-dihydro-2*H*-pyran (**7**)

Malonaldehyde (propanedial) (**1**) can be formed *in vivo* by the oxidation of unsaturated lipids,[1] as a by-product of the biosynthesis of prostanoids,[2] and by attack of radicals on deoxyribose.[3] It is mutagenic[4] and is implicated in the ageing process.[5] Consequently, there is considerable interest in the reactions of malonaldehyde with DNA and proteins. Recently, adducts of malonaldehyde with nucleosides[6–8] and DNA[9] have been described. These were 1:1 adducts[6–8] [*e.g.* compounds (**2**) and (**3**)], 2:1 adducts[7] [*e.g.* (**4**)], and 3:1 adducts[8] [*e.g.* (**5**)].



In connection with our studies of cyclic nucleic acid adducts[10] we have investigated the oligomerisation chemistry of malonaldehyde in aqueous solution. It has long been known that malonaldehyde oligomerises in water, but despite many studies no conclusive structural evidence for the oligomers has been presented.[11–13] However, condensation products of malonaldehyde are known to react with DNA[4] and proteins.[14] To help assess the relevance of the oligomers to the toxicology of malonaldehyde, we have attempted to obtain these compounds pure and to characterise them. We have found that malonaldehyde undergoes a relatively rapid dimerisation and trimerisation at 20 °C in water at pH 4.2. We report herein the structures of the compounds formed and some preliminary information pertaining to their chemistry.

A 1M solution (pH 4.2) of malonaldehyde[15] in water was monitored by ^{1}H n.m.r. spectroscopy and this showed the formation of (*E*)-2-formylpent-3-ene-1,5-dial (**6**) and 4-(1,3-dioxopropan-2-yl-5-formyl-2-hydroxy-3,4-dihydro-2*H*-pyran (**7**) (ratio 1:4) at 20 °C. These compounds had appeared within 15 min and all the malonaldehyde had been consumed after 3 days. Only after 25 days were additional products present in appreciable concentrations. From preparative scale reactions compounds (**6**) and (**7**) were isolated as metal salts by fractional crystallisation and chromatography.†

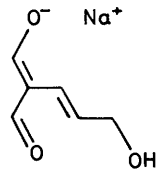


† Reactions initially contained either the potassium or sodium salt of malonaldehyde (0.3M in water). The pH was adjusted to 4.2 (N.B. pK_a of malonaldehyde = 4.46) by addition of dilute hydrochloric acid. After disappearance of the malonaldehyde (19 h at 37 °C) the solution was concentrated and sodium chloride was precipitated by addition of methanol. The filtrate was concentrated and the residue was redissolved in methanol. This solution when cooled to 0 °C and diluted with dichloromethane gave a precipitate of compound (**7**). This was further purified by recrystallisation from aqueous acetone to give a white solid, m.p. 161—162 °C (potassium salt). The filtrate from the initial precipitation of (**7**) was concentrated and the residue was chromatographed on silica (elution of the orange band with 15% methanol in dichloromethane) to give compound (**6**). This was recrystallised from methanol–dichloromethane to give an orange solid (decomposed without melting on heating). Both compounds (**6**) and (**7**) contained one sodium ion (or potassium ion, when the potassium salt of malonaldehyde was used as starting material) per molecule. For (**6**): $\delta_H(D_2O)$ 7.00 (1 H, dd, *J* 8.7 and 15.4 Hz, 4-H), 7.54 (1 H, d, *J* 15.4 Hz, 3-H), 8.95 (2 H, br s, 1-H and 2-CHO) and 9.25 (1 H, d, *J* 8.7 Hz). The ^{1}H n.m.r. spectrum of (**6**) in acidic D_2O gradually simplifies because of exchange of 4-H; $\delta_C(CD_3OD)$ 117.3 (C-3), 122.8 (C-4), 149.7 (C-2), 191.9 (C-1 and 2-CHO), and 198.8 (C-5); $\lambda_{max.}(H_2O)$ 275 (ε 5 000), 350 (6 500) and 476 nm (300); $\lambda_{max.}$(acidic H_2O) 260 (5 000) and 295 nm (4 300); $\nu_{max.}$(KBr) 1 655 cm^{-1}; *m/z* (f.a.b.) 149 (corresponds to protonated monosodium salt). For (**7**): δ_H 1.94 (2 H, dd, *J* 4 and 6 Hz, 2 × 3-H), 3.70 (1 H, t, *J* 6 Hz, 4-H), 5.50 (1 H, t, *J* 4 Hz, 2-H) and 8.20 (4 H, br s, 6-H, 5-CHO, and OCH=CCHO); $\lambda_{max.}(H_2O)$ 250 (ε 20 100) and 270 nm (12 200); $\lambda_{max.}$(acidic water) 251 nm (31 900); $\nu_{max.}$(KBr) 1 615 and 1 648 cm^{-1}; *m/z* (f.a.b.) 275 [corresponds to protonated dipotassium salt (**11**)].

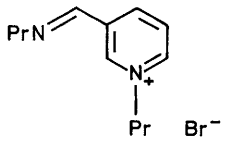
Structure (**6**) follows from spectroscopic data* and the finding that addition of sodium borohydride to a solution of (**6**) in D_2O showed immediate formation of an alcohol assigned structure (**8**): δ_H 4.26 (2 H, d, *J* 6 Hz, 2 × 5-H), 6.44 (1 H, d, *J* 16 Hz, 3-H), 6.65 (1 H, dt, *J* 6 and 16 Hz, 4-H), 8.64 (2 H, br s, 1-H and 2-CHO).

Structure (**7**) follows from spectroscopic data,* including comparison with data for compound (**10**)† [prepared by the neutralisation with NaOH of a partial acidic hydrolysis of 1,1,3,3-tetramethoxypropane (*i.e.* condensation of 1 molecule of 3,3-dimethoxypropanal with 2 molecules of malonaldehyde, *cf.* ref. 16)]. The ^1H n.m.r. spectrum of compound (**7**) is pH dependent. Raising the pD of a solution of (**7**) in D_2O to 12 showed its conversion into the dianion (**11**), in which the aldehyde function can be trapped by borohydride reduction. Equilibration of (**7**) with (**11**) explains why the ^1H n.m.r. spectrum* of compound (**7**) shows apparent equivalence of 6-H, 5-CHO, and the protons in the OCH=CCHO group. Ring-opening of (**7**) to (**11**), can be followed by one of two energetically identical modes of cyclisation. The *cis* stereochemistry assigned to (**7**) is consistent with its acid-catalysed cyclisation to 2,8-dioxabicyclo[3.3.1]nona-3,6-diene-4,6-dicarbaldehyde (**12**). Thus, adjustment to pH 2 of a solution of (**7**) or (**10**), and extraction with dichloromethane gave the acetal (**12**), m.p. 148 °C, $\delta_H(CDCl_3)$ 1.49 (2 H, dd, *J* 2 and 2 Hz, 2 × 9-H), 3.68 (1 H, dt, 2 and 5 Hz, 5-H), 5.77 (1 H, dt, 2 and 5 Hz, 1-H), 6.94 (2 H, s, 3-H and 7-H), and 8.88 (2 H, s, 2 × CHO).†

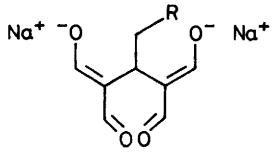
The 2:1 and 3:1 adducts described[7,8] from reactions of malonaldehyde and nucleosides can either be explained as products of a stepwise condensation of a nucleoside with 2 (or 3) molecules of malonaldehyde, or could arise from the condensation of (**6**) or possibly (**7**) with a nucleoside. The rate of self-condensation of malonaldehyde is competitive with its rate of condensation with nucleosides.[8,17] We are therefore investigating reactions of (**6**) and (**7**) with nucleosides and oligonucleotides, although no experimentation is required to conclude that the structures and mechanisms proposed[8] for the 3:1 adducts of malonaldehyde with nucleosides are unlikely! The potential ability of (**6**) to crosslink oligonucleotides and/or proteins was explored in a model experiment. Thus, reaction of 1.35M (**6**) with propylamine hydrobromide (2 mol equiv.) in D_2O at pD 10.2 gave the propylamine imine of *N*-propyl-3-formylpyridinium bromide (**9**) within 30 min at 20 °C. This substance† was separately prepared by treating propylamine with *N*-propyl-3-formylpyridinium bromide† (obtained by treating pyridine-3-carbaldehyde with 1-bromopropane).



(**8**)

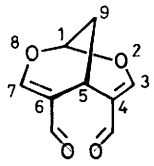


(**9**)



(**10**) R = $CH(OMe)_2$

(**11**) R = CHO



(**12**)

* See footnote on p. 2168.

† Satisfactory spectroscopic and analytical data were obtained for this compound.

Acknowledgements

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