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Reaction of Malondialdehyde with Amine Neurotransmitters. Formation and Oxidation Chemistry of Fluorescent 1,4-Dihydropyridine Adducts.

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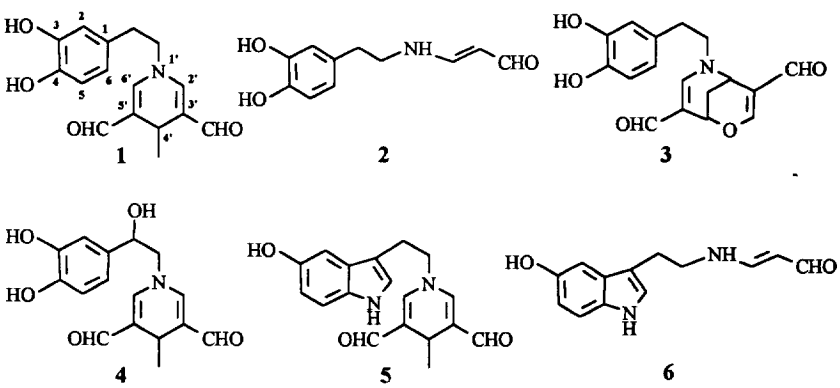
Abstract. Under physiologically relevant conditions, malondialdehyde reacts smoothly with amine neurotransmitters, i.e. dopamine, norepinephrine and serotonin, to give the fluorescent dihydropyridines **1**, **4** and **5**, respectively, as the relatively most abundant products. Small amounts of enaminal derivatives, such as **2** and **6**, could also be obtained in the reactions with dopamine and serotonin. Oxidation of **1** with hydrogen peroxide/peroxidase leads to a complex pattern of unstable products, the major of which has been isolated and identified as the o-quinone epoxide **7**. Similar oxidation of **4** and **5** affords mainly the dihydropyridine **8** and the 4,4'-biindolyl **9**, respectively. These results provide new clues to the role of malondialdehyde in neuronal degeneration and lipofuscin formation.

Since the free radical theory of ageing was first put forward by Harman in 1956,[1] a massive body of experimental evidence has accrued suggesting that oxidative stress-induced lipid peroxidation is a main pathogenic mechanism of degradative changes in the central nervous system associated with age[2] or with certain pathological conditions, *e.g.* Parkinson's disease[3] and senile dementia of the Alzheimer's type.[4] Besides modifications of membrane structure and fluidity, or disturbances of calcium homeostasis, a major consequence of lipid peroxidation is the overproduction of a range of diffusible degradation products, including notably malondialdehyde.[5] This latter has been the subject of much interest because of its possible implication in various cytotoxic and mutagenic processes, as well as in the genesis of the fluorescent age-pigments lipofuscins.[6]

Several studies have been aimed at correlating the biological activities of malondialdehyde with its ability to modify and cross-link macromolecular cellular components.[5] Polypeptides and proteins were shown to be affected *via* formation of covalent adducts of the aldehyde with the ε-amino groups of lysine residues,[7,8] whereas nucleic acids were found to lose their template activity following chiefly modification at guanine moieties.[9,10] Comparatively little is known on the reaction of malondialdehyde with other possible biochemical targets of low molecular weight, which are likewise central for neurone functioning and interaction with neighbouring cells. In connection with our interest in the mechanisms of neuronal degeneration, we have recently been prompted to look at amine neurotransmitters as targets of

malondialdehyde.[11] In this paper we report the structures of the major adducts derived from dopamine, norepinephrine and serotonin, and their oxidation chemistry under biologically relevant conditions.

In aqueous buffer at pH 6.0 and at room temperature, dopamine reacted smoothly with malondialdehyde (1:1 to 1:5 molar ratio) to give the yellow, fluorescent 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde **1**, along with the colourless enaminal **2** in moderate yields. When a large excess of malondialdehyde was used, and/or the aldehyde solution was left for some time prior to incubation with dopamine, the oxaazabicyclononadiene **3** was also obtained.[11] The germane catecholamine norepinephrine displayed comparable reactivity with malondialdehyde to give **4** as the chief isolable product, whereas 5-hydroxytryptamine (serotonin) afforded the dihydropyridine **5** along with the enaminal **6**. In spite of some fluctuations in the yields, depending on the concentration and molar ratio of the reagents, the dihydropyridine adducts were generally formed in higher yields compared to enaminals. When the reaction of malondialdehyde with amine neurotransmitters was conducted at higher pH, e.g. 7.4, formation of the above adducts was slightly reduced, without substantial modification of the product patterns.



1 2 3 4 5 6

Formation of 4-methyl-1,4-dihydropyridine-3,5-dicarboxaldehydes by reaction of malondialdehyde with alkylamines and aminoacid derivatives is known,[12,13] and has been shown to involve the intermediacy of enaminal adducts, analogous to **2** and **6**. Partial hydrolytic cleavage of such enaminals accounts for the liberation of some acetaldehyde leading eventually to the dihydropyridine ring *via* a Hantzsch-type reaction.[12]

As expected, the dihydropyridine adducts were markedly susceptible to oxidation, especially by the hydrogen peroxide/peroxidase system, which is believed to be critical in oxidative stress-induced cell damage.[14] When **1** was allowed to oxidise under such conditions, it afforded in the early stages a major fluorescent product which slowly decomposed in the reaction mixture. This was obtained as a yellow oil by preparative TLC of the ethyl acetate-extractable fraction. The compound exhibited UV and fluorescence spectra closely similar to those of **1**. The FT-IR spectrum displayed two strong bands at 1665 and 1602 cm^{-1}. Scrutiny of the ^1H-NMR spectrum revealed resonances for a dihydropyridine ring linked to an ethylene chain, the distal methylene protons being split apparently by an adjacent asymmetric centre. Two doublets at δ 7.29 and 6.15 (1H each) were also present, along with a singlet (1H) for a deshielded aliphatic proton at δ 3.78. Salient features of the ^{13}C-NMR spectrum included, besides the dihydropyridine ring signals, resonances at δ 55.89 (quaternary carbon), 62.61, 131.01 and 149.10 (CH carbons, DEPT). These latter correlated with the

protons at δ 3.78, 6.15 and 7.29, respectively, as evidenced by 2D carbon-proton shift correlation experiments. Taken together, these data suggested the structure of the o-quinone epoxide **7**,[15] arising by nucleophilic addition of hydrogen peroxide to the o-quinone of **1** (Figure 1). Consistent with this formulation is the marked instability of the compound to acids and dilute alkali, and its susceptibility to reduction with sodium borohydride, without giving isolable products. Although commonly implicated in the reaction of hydrogen peroxide with o-quinones, to the best of our knowledge only a few simple o-quinone epoxides have been isolated and characterised.[16]

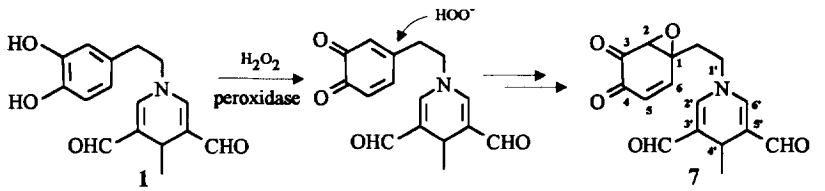


Figure 1

The dihydropyridine **4** was also facile to oxidation with hydrogen peroxide/peroxidase, leading to a single fluorescent product in moderate yield. This was identified as 4-methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (**8**) by straightforward spectral analysis. The remainder of the reaction mixture was accounted for by chromatographically ill-defined products. In separate experiments, evidence was obtained that **8** is stable under the oxidising conditions adopted in the present study, which would rule out any significant decomposition of the dihydropyridine ring in the oxidation of **4** and its congeners.[17]

Formation of the dihydropyridine **8** by oxidation of **4** requires conversion of the catechol ring to the corresponding o-quinone, whose subsequent fate is possibly driven by the presence of the hydroxyl group on the adjacent carbon, favouring the generation of a quinone methide intermediate. A plausible sequence of reactions accounting for the formation of **8** is shown in Figure 2.

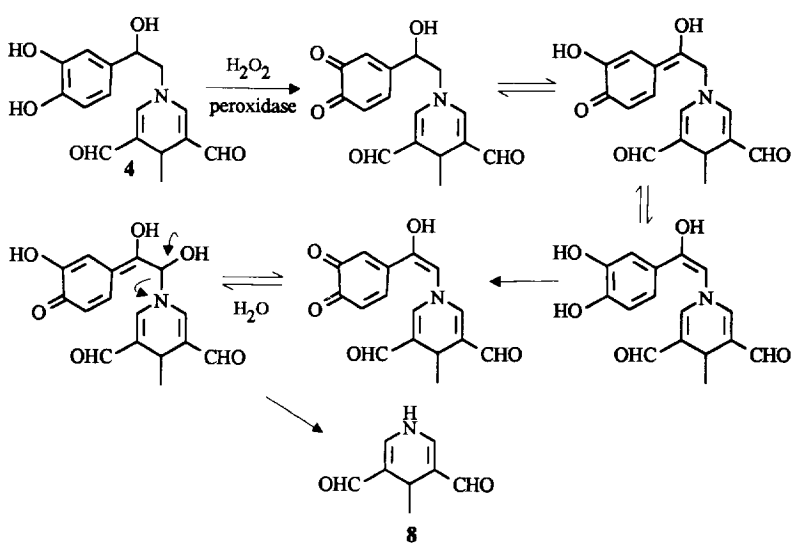


Figure 2

Oxidation of **5** with hydrogen peroxide/peroxidase also gave a complex mixture of fluorescent products. One of these, which was relatively more abundant, could be isolated and was identified as the symmetric dimer **9**. The site of coupling was readily apparent from the lack of the H-4 signal in the ^{1}H-NMR spectrum. Formation of dimer **9** is well in line with the known proclivity of the 5-hydroxyindole system to react at the 4-position,[18] and probably results from nucleophilic attack of the parent compound to a transient quinoneimine-type intermediate, as outlined in Figure 3.

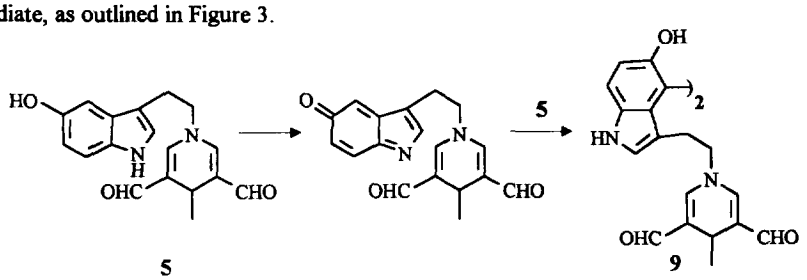


Figure 3

The characterisation of compounds **7-9** suggests that the oxidation behaviour of fluorescent dihydropyridine adducts between malondialdehyde and amine neurotransmitters is governed by the inherent reactivity of the structural moiety linked to the dihydropyridine ring. Of particular interest is the mode of decomposition of the catecholamine adducts **1** and **4**, in which preclusion of the normal intramolecular cyclisation pathway[19] allowed isolation of an o-quinone epoxide intermediate, and caused partial loss of the nitrogen-containing moiety, respectively.

In conclusion, the results of this study point to a possible role of amine neurotransmitters as targets of malondialdehyde formed by lipid peroxidation in neurodegenerative processes. Occurrence of similar reactions in vivo would expectedly lead to a range of oxidisable adducts that can not be metabolised by monoamine oxidases, and that may chemically interact with crucial cellular structures following decomposition in an oxidising milieu. Investigation of the biological activities of the new compounds may provide further links to correlate lipid peroxidation with increased lipofuscin accumulation and altered neurotransmission in ageing and in neurodegenerative disorders.

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EXPERIMENTAL

UV spectra were performed with a Perkin-Elmer Lambda 7 spectrophotometer. Fluorescence spectra were recorded on a Perkin Elmer LS 50 B spectrofluorimeter. FT-IR spectra were determined on a Perkin Elmer model 1760-X spectrophotometer. ^{1}H-NMR (270 and 400 MHz) and ^{13}C-NMR (67.9 and 100 MHz)

spectra were carried out on Bruker AC 270 and WM 400 spectrometers. Tetramethylsilane was used as reference standard. Electron impact mass spectra (EI-MS) were determined on a Fisons Trio 2000 apparatus. Main fragmentation peaks are reported with their relative intensities (percent values are in brackets). TLC was carried out on pre-coated silica gel F-254 plates from Merck. The chromatograms were examined by UV irradiation at 254 and at 366 nm. HPLC analyses were carried out on a Gilson apparatus equipped with a Gilson mod. 117 UV detector set at 280 nm. RP18 Spherisorb S50DS2 (4.0 x 250 mm, Phase Separation Ltd.) or Econosil C-18 (10 x 250 mm, Alltech) columns were used for analytical and preparative purposes, with flow rates of 1 mL/min and 6 mL/min, respectively.

Horseradish peroxidase (donor:H_2O_2 oxidoreductase EC 1.11.1.7) type II (220 U/mg, RZ E_{430}/E_{275}=2.0) was from Sigma. Dopamine hydrochloride, (±)-norepinephrine hydrochloride, serotonin creatinine sulphate monohydrate, hydrogen peroxide (30%, stabilised) were purchased from Aldrich. Malondialdehyde was used either as a solution (about 0.5 M) obtained by acid hydrolysis of 1,1,3,3-tetramethoxypropane[20] or as the sodium salt.[8] Glass distilled and deionised water was used for preparation of all solutions.

Reaction of malondialdehyde with amine neurotransmitters. General procedure.

Reaction of malondialdehyde with amine neurotransmitters was typically carried out at room temperature by adding a freshly prepared solution of the aldehyde to a solution of the amine in 0.5 M acetate buffer, pH 6.0, at the desired concentration. No substantial difference in the product patterns was observed using the sodium salt of malondialdehyde, as apparent from TLC and HPLC. Experiments at higher pH, e.g. 7.4, were performed in 0.1 M phosphate buffer.

For preparative purposes, fresh malondialdehyde (20 mL) was added to a solution of the amine (about $2x10^{-3}$ mol) in 0.5 M acetate buffer, pH 6.0 (160 mL) at room temperature. The reaction was monitored by HPLC using a linear gradient from 0.1 M formic acid to 0.1 M formic acid/acetonitrile 1:1 v/v over 30 min. After 4 h, or when most of the starting material had disappeared, the mixture was repeatedly extracted with ethyl acetate. The organic layers were combined, dried over sodium sulphate and evaporated to dryness, and the residue was chromatographed as detailed below.

Isolation of compounds 1-3.

The yellow oily residue obtained by reaction of malondialdehyde with dopamine was chromatographed on 1 mm silica gel plates (eluent acetone-benzene 6:4 v/v) to afford a number of UV absorbing bands. A relatively less polar, blue fluorescent band (R_f=0.77) consisted of pure **1** (yellow amorphous solid, about 30% yield based on reacted dopamine). UV (CH_3OH): λ_{max} (log ε) 236 (4.3), 266 (4.0), 398 (3.9) nm; λ_{em}=460 nm (λ_{ex} = 398 nm, CH_3OH). EI-MS m/z: 287 (10, M^+), 272 (20), 205 (25), 163 (100), 149 (83), 137 (60). Exact mass calcd. for $C_{16}H_{17}NO_4$: 287.1158; found: 287.1166. ^{1}H-NMR (CD_3OD) δ (ppm): 9.07 (1Hx2, s, CHO), 6.90 (1H x 2, s, H-2', H-6'), 6.70 (1H, d, J=8.1 Hz, H-5), 6.66 (1H, d, J=2.2 Hz, H-2), 6.53 (1H, dd, J=8.1, 2.2 Hz, H-6), 3.73 (1H, q , J=6.5 Hz, H-4'), 3.73 (2H, t, J=6.5 Hz, CH_2), 2.83 (2H, t, J=6.5 Hz, CH_2), 0.96 (3H, d, J=6.5 Hz, CH_3).

The band at R_f=0.46 afforded **2** (E isomer, about 15% yield) as colourless oil. UV (H_2O) λ_{max}=280 nm. EI-MS m/z 207 (15, M^+), 123 (35), 84 (100). Exact mass calcd. for $C_{11}H_{13}NO_3$: 207.0892; found: 207.0886. ^{1}H-NMR (CD_3OD) δ (ppm): 8.81 (1H, d, J=8.8 Hz, CHO), 7.36 (1H, d, J=12.8 Hz, CH=), 6.70

(1H, d, J=8.0 Hz, H-5), 6.66 (1H, d, J=2.0 Hz, H-2), 6.53 (1H, dd, J=8.0, 2.0 Hz, H-6), 5.30 (1H, dd, J=12.8, 8.0 Hz, CH=), 3.42 (2H, t, J=6.8 Hz, CH_2), 2.72 (2H, t, J=6.8 Hz, CH_2). Very minute amounts of the Z isomer were occasionally detected as an impurity: δ (ppm): 8.63 (1H, d, J=9.0 Hz, CHO), 7.20 (1H, d, J=9.8 Hz, CH=), 5.27 (1H, dd, J=9.8,9.0 Hz, CH=). The other resonances are obscured by overlapping peaks.

Under the above conditions, formation of compound **3** (band at R_f=0.5 on TLC, eluent acetone-benzene 6:4) was negligible. Significant yields (about 10%) could be obtained using higher concentrations of malondialdehyde and/or leaving the malondialdehyde solution at room temperature for some time prior to incubation with dopamine. Spectral data for **3** have been reported previously.[11]

Isolation of compound 4.

The residue obtained from reaction of norepinephrine with malondialdehyde was chromatographed on 1 mm silica gel plates (eluant: acetone-cyclohexane 7:3 v/v). Elution of the band at R_f=0.5 gave **4** (about 20% yield) as a yellow oil. UV (CH_3OH) λ_{max}=232, 265, 384 nm. λ_{em}=457 nm (λ_{ex} = 384 nm, CH_3OH). EI-MS m/z: 303 (8, M^+), 288 (15), 285 (24), 270 (100). Exact mass calcd. for $C_{16}H_{17}NO_5$: 303.1107; found: 303.1102. ^{1}H-NMR (Acetone-d_6), δ (ppm): 9.21 (1Hx2, d, J=1.3 Hz, CHO), 7.09 and 7.06 (1H each, d, J=1.3 Hz, H-2' and H-6'), 6.94 (1H, d, J=1.8 Hz, H-2), 6.80 (1H, d, J=8.1 Hz, H-5), 6.75 (1H, dd, J=8.1, 1.8 Hz, H-6), 4.87 (1H, dd, J=6.8, 4.6 Hz, CHOH), 3.76 (1H, q, J=8.1 Hz, H-4'), 3.78 and 3.69 (1H, dd, J=13.5, 4.6 Hz and 1H, dd, J=13.5, 6.8 Hz, CH_2), 0.99 (3H, d, J=8.1 Hz, CH_3).

Isolation of compounds 5 and 6

The yellow residue obtained after work up of the reaction mixture of malondialdehyde with serotonin was chromatographed on 1 mm silica gel plates (eluent acetone-cyclohexane 7:3 v/v) to give a major band (R_f=0.75). This was re-chromatographed on 0.5 mm silica gel plates (eluent ethyl acetate-chloroform 95:5 v/v) to afford two bands.

The less polar band (R_f=0.50) gave compound **5** (about 30% yield) as a yellow solid. UV (CH_3OH) λ_{max} (log ε) 269 (4.2), 310 (shoulder, 3.8) 389 (3.9) nm. λ_{em}=463 nm (λ_{ex} = 389 nm, CH_3OH). EI-MS (m/z): 310 (12, M^+), 295 (28), 294 (35), 160 (100). Exact mass calcd. for $C_{18}H_{18}N_2O_3$: 310.1313; found: 310.1321. ^{1}H-NMR (CD_3OD), δ (ppm): 9.00 (1Hx2, s, CHO), 7.18 (1H, dd, J=8.5, 0.5 Hz, H-7), 6.98 (1H, s, H-2), 6.94 (1H, dd, J=2.2, 0.5 Hz, H-4), 6.80 (1Hx2, s, H-2', H-6'), 6.68 (1H, dd, J=8.5, 2.2 Hz, H-6), 3.82 (2H, t, J=5.6 Hz, CH_2), 3.68 (1H, q, J=6.5 Hz, H-4'), 3.07 (2H, t, J=5.6 Hz, CH_2), 0.96 (3H, d, J=6.5 Hz, CH_3).

The second band (R_f=0.25) gave **6** (E isomer, about 10% yield) as a colourless solid: UV (CH_3OH): λ_{max}= 280 nm. EI-MS: m/z 230. Exact mass calcd. for $C_{13}H_{14}N_2O_2$: 230.1052; found: 230.1056. ^{1}H-NMR (CD_3OD) δ (ppm): 8.88 (1H, d, J=8.8 Hz, CHO), 7.40 (1H, d, J=12.6 Hz, CH=), 7.15 (1H, d, J=8.6 Hz, H-7), 7.05 (1H, s, H-2), 6.91 (1H, d, J=1.8 Hz, H-4), 6.66 (1H, dd, J=8.6, 1.8 Hz, H-6), 5.36 (1H, dd, J=12.6, 8.8 Hz, CH=), 3.40 (2H, t, J=7.0 Hz, CH_2), 2.97 (2H, t, J=7.0 Hz, CH_2). Very minute amounts of the Z isomer were occasionally detected: δ (ppm): 8.56 (1H, d, J=8.9 Hz, CHO), 6.98 (1H, s, H-2), 6.94 (1H, d, J=1.8 Hz, H-4), 5.25 (1H, dd, J=9.8, 8.9, CH=), 3.52 (2H, t, J= 7.0 Hz, CH_2), 2.92 (2H, t, J= 7.0 Hz, CH_2). The other resonances are obscured by overlapping peaks.

*Oxidation of **1** with peroxidase/hydrogen peroxide. Isolation of compound **7**.*

Peroxidase (4 mg in 4 mL of water) was added to a solution of **1** (200 mg) in 0.1 M phosphate buffer, pH 7.0 (200 mL), followed by conc. hydrogen peroxide (150 μL) under stirring at room temperature. After about 15 min, the mixture was acidified to pH 5.0 with 1 M HCl and extracted with ethyl acetate (4 x 60 mL). The organic layers were concentrated at room temperature and carefully chromatographed on 0.5 mm silica gel plates (eluant: acetone-cyclohexane 7:3 v/v). Elution of the band at R_f=0.6 gave **7** as a yellow oil (about 10% yield) which slowly decomposed in solution. UV (CH_3OH): λ_{max}= 234, 258, 384 nm. λ_{em}=460 nm (λ_{ex} = 384 nm, CH_3OH). FT-IR ($CHCl_3$): 1665, 1602 cm^{-1}. EI-MS: no detectable molecular ion peak at m/z 301 could be obtained under a variety of conditions; fragmentation peaks were observed at m/z 285 (3), 257 (9), 256 (9), 243 (6), 229 (6), 213 (12), 199 (7), 185 (14), 167 (30), 151 (25), 149 (100), 136 (24), 129 (32). ^1H NMR (Acetone-d_6) δ (ppm): 9.25 (1Hx2, s, CHO), 7.29 (1H, d, J=10.0 Hz, H-6), 7.22 (1Hx2, s, H-2' and H-6'), 6.15 (1H, d, J=10.0 Hz, H-5), 3.89 (2H, t, J=7.1 Hz, CH_2), 3.78 (1H, q, J=6.5 Hz, H-4'), 3.78 (1H, s, H-2), 2.49 and 2.19 (1H each, dt, J=14.0, 7.1 Hz, CH_2), 0.99 (3H, d, J=6.5 Hz, CH_3). ^{13}C NMR (Acetone-d_6) δ (ppm): 193.52 (C-3 or C-4), 189.57 (CHO) 149.10 (C-6), 148.01 and 147.70 (C-2' and C-6'), 131.01 (C-5), 129.8 (C-3' and C-5'), 62.61 (C-2), 55.89 (C-1), 51.60 (CH_2), 34.63 (CH_2), 23.44 (C-4'), 22.29 (CH_3). The resonance of one of the carbonyl groups could not be detected, probably owing to the gradual decomposition of the sample during acquisition of the ^{13}C-NMR spectrum.

*Oxidation of **4** with peroxidase/hydrogen peroxide. Isolation of compound **8**.*

Oxidation of **4** (200 mg) was carried out essentially as described for compound **1**. After about 15 min, the mixture was exhaustively extracted with ethyl acetate, the organic phases were combined and evaporated to dryness. The yellow residue was chromatographed on 0.5 mm silica gel plates (eluant: chloroform-methanol 8:2 v/v) and the fluorescent band at R_f=0.6 was collected to give **8** as a yellow oil (about 10% yield). UV (CH_3OH): λ_{max}=227, 252, 374 nm. λ_{em}=444 nm (λ_{ex} = 374 nm, CH_3OH). FT-IR ($CHCl_3$): 1667, 1605, 1474 cm^{-1}. EI-MS m/z: 151 (45, M^+), 137 (40), 136 (100). Exact mass calcd. for $C_8H_9NO_2$: 151.0631; found: 151.0625. ^1H-NMR (CD_3OD), δ (ppm): 9.21 (1Hx2, s, CHO), 7.16 (1Hx2, s, H-2 and H-6), 3.85 (1H, q, J=6.5 Hz, H-4), 1.04 (3H, d, J=6.5 Hz, CH_3).

*Oxidation of **5** with peroxidase/hydrogen peroxide. Isolation of compound **9**.*

Peroxidase (8 mg) in water (4 mL) was added to a solution of **5** (100 mg) in 0.1 M phosphate buffer, pH 7.0 (200 mL), followed by 0.8 M hydrogen peroxide (1.2 mL) under stirring at room temperature. After about 30 min the mixture was extracted with ethyl acetate (4x60 mL), the organic layers were combined and evaporated to dryness to give an oily residue. Preparative HPLC (eluent: 0.1 M formic acid-acetonitrile 7:3 v/v) furnished **9** as a yellow amorphous solid (yield about 18%). UV (CH_3OH): λ_{max} (log ε) 268 (4.1), 310 (shoulder, 3.8), 392 (3.9) nm. λ_{em}=463 nm (λ_{ex}=392 nm, CH_3OH). EI-MS m/z: 618 (M^+). Exact mass calcd. for $C_{36}H_{34}N_4O_6$: 618.2470; found: 618.2474. ^1H-NMR (DMSO-d_6), δ (ppm): 9.08 (1Hx4, s, CHO), 7.23 (1Hx2, d, J=8.5 Hz, H-7), 7.05 (1Hx2, s, H-2), 6.82 (1Hx2, d, J=8.5 Hz, H-6), 6.43 and 6.37 (1Hx2 each, s, H-2', H-6'), 3.50 (1Hx2, q, J=6.5 Hz, H-4'), 3.09 (2H, t, J=5.6 Hz, CH_2), 2.92 (2H, t, J=5.6 Hz, CH_2), 2.32 (2H, t, J=5.6 Hz, CH_2), 2.18 (2H, t, J=5.6 Hz, CH_2), 0.81 (3Hx2, d, J=6.5 Hz, CH_3).

REFERENCES AND NOTES

1. Harman, D. *J. Gerontol.* **1956**, 11, 298-300.
2. Harman, D. *Proc. Natl. Acad. Sci. USA* **1981**, 78, 7124-7128.
3. Dexter, D.T.; Cater, C.J.; Wells, F.R.; Javoy-Agid, F.; Agid, Y.; Lees, A.; Jenner, P.; Marsden, C.D. *J. Neurochem.* **1989**, 52, 381-389.
4. Harman, D. Nutrition and Age Pigment: Pathogenesis of Senile Dementia of the Alzheimer's type. In: *Advances in Age Pigments Research*, 64. Aloj Totaro, E.; Glees, P.; Pisanti, F.A., eds., Pergamon Press: Oxford, 1987; pp. 75-84.
5. Esterbauer, H.; Schaur, R.J.; Zollner, H. *Free Rad. Biol. Med.* **1991**, 11, 81-128
6. Sohal, R.S. *Age Pigments.* Elsevier/North Holland: Amsterdam, 1981.
7. Chio, K.S.; Tappel, A.L. *Biochemistry*, **1969**, 8, 2821-2826.
8. Nair, V., Vietti, D.E., Cooper, C.S. *J. Am. Chem. Soc.* **1981**, 103, 3030-3036.
9. Summerfield, F.W., Tappel, A.L. *Anal. Biochem.* **1984**, 143, 265-271.
10. Kikugawa, K.; Taguchi, K.; Maruyama, T. *Chem. Pharm. Bull.* **1987**, 35, 3364-3369.
11. d'Ischia, M.; Costantini, C.; Prota, G. *Tetrahedron Lett.* **1993**, 34, 3921-3924.
12. Nair, V.; Offerman, R.J.; Turner, G.A.; Pryor, A.N.; Baenzinger, N.C. *Tetrahedron* **1988**, 44, 2793-2803.
13. Kikugawa, K.; Machida, Y.; Kida, M.; Kurechi, T. *Chem. Pharm. Bull.* **1981**, 29, 3003-3011.
14. Halliwell, B. *J. Neurochem.* **1992**, 59, 1609-1623
15. Epoxide carbons in the ^{13}C-NMR spectrum usually resonate at slightly higher fields compared to those belonging to other oxygenated systems, e.g.1,2-diols. See for example: Wehrli, F.W.; Nishida, T. *Fortschr. Chem. Org. Naturst.* **1979**, 36, 1-229.
16. See for example: Albert, K.; Schneider, H.-P.; Koch, D.; Rieker, A. *Z. Naturf.* **1978**, 33b, 100.
17. The resistance of the dihydropyridine-3,5-dicarboxaldehyde ring to oxidation with hydrogen peroxide was also apparent from separate experiments carried out on similar adducts which had been prepared for other purposes.
18. Napolitano, A.; d'Ischia, M.; Prota, G. *Tetrahedron* **1988**, 44, 7265-7270.
19. Tse, D.C.S.; Mc Creery, R.L.; Adams, R.N. *J. Med. Chem.* **1976**, 19, 37-40.
20. Bertz, S.H. *J. Org. Chem.* **1985**, 50, 3585-3592.

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