528. *Hydroxypolyporic Acids and Related Compounds as Antioxidants for Linoleic Acid and Methyl Linoleate.*

By G. J. Bennett and N. Uri.

Some polyhydroxypolyporic acids have been prepared and tested as antioxidants for linoleic acid and methyl linoleate: their mode of action is discussed. 3′,4′,5′-Trihydroxy-4″-t-butylpolyporic acid proved to be outstanding.

It is generally assumed that this autoxidation involves a chain reaction of the type:

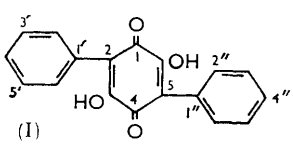
$$R\cdot + O_2 \longrightarrow RO_2\cdot$$
$$RO_2\cdot + RH \longrightarrow RO{\cdot}OH + R\cdot$$

The mechanism of antioxidation, which has been discussed elsewhere,[1] is postulated as mainly due to the chain-breaking reaction:

$$RO_2\cdot + AOH \longrightarrow RO{\cdot}OH + AO\cdot$$

where AOH represents the phenolic antioxidant. The AO· radical should not be a chain-carrier and must possess a high degree of resonance stabilisation. Some of the applied aims of this study have recently been described elsewhere.[2]

The dihydroxyquinones investigated in this work include embelin (2,5-dihydroxy-3-undecyl-1,4-benzoquinone) and the related 2,5-dihydroxy-3,6-diundecyl-1,4-benzoquinone, and derivatives of the related polyporic acid (I). Our syntheses were usually effected by the method described by Akagi [3e] for "leucomelone." 2,5-Dichloro-1,4-benzoquinone and an *N*-nitrosoacetanilide gave the dichloromonoarylquinone, the other aryl group being then introduced by diazo-coupling. Hydrolysis of the dichloroterphenylquinone gave the corresponding polyporic acid which, if necessary, is finally demethylated to give a hydroxypolyporic acid. (Direct demethylation where possible was preferred to reductive demethylation, followed by oxidation.)



(I)

Antioxidant activities were examined for pure linoleic acid, for pure methyl linoleate, for aqueous emulsions of the latter, and in 0·2M-solutions in ethyl benzoate with ferrous phthalocyanine as catalyst. Protection factors were defined as $(t_a - t_o)_4/t_o$, where t_a was the time required to reach a peroxide value of 25 (millimoles of peroxide per g. of fatty acid or ester) in the presence of antioxidant at a 2×10^{-4}M-concentration, and t_o was the

[1] Uri, "Autoxidation and Antioxidants," ed. W. O. Lundberg, Interscience Publ., Inc., New York, 1961, Vol. I, pp. 133—168.
[2] Bennett and Uri, *Nature*, 1961, **192**, 354.
[3] Akagi, *J. Pharm. Soc. Japan*, 1942, **62**, (*a*) 129, (*b*) 191, (*c*) 195, (*d*) 199, (*e*) 202.

TABLE 1.

Protection factors with various 2,5-dihydroxyquinones in linoleic acid.

Pr gallate	2·2	4′,4″-Dimethoxypolyporic acid	0·6
Embelin	2·5	3′,4′,5′-Trimethoxypolyporic acid	0·8
2,5-Dihydroxy-3,6-diundecyl-1,4-benzoquinone	<0·1	3′,4′,5′-Trihydroxypolyporic acid	25
Polyporic acid	<0·1	Atromentin (4′,4″-dihydroxypolyporic acid)	4·0
4′-Methoxypolyporic acid	0·4	Leucoatromentin	6·5
		3′,4′,5′-Trihydroxy-4″-t-butylpolyporic acid	60

corresponding time in absence of an antioxidant. Table 1 presents data for pure linoleic acid (precision about ±20%). Results for the efficient antioxidants in methyl linoleate are presented in Table 2.

TABLE 2.

Protection factors with various 2,5-dihydroxyquinones in methyl linoleate.

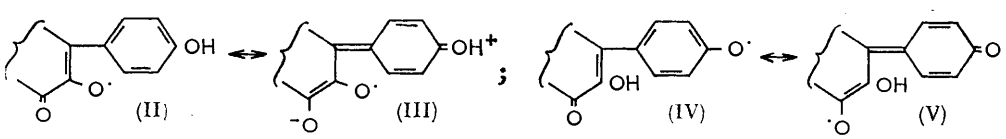
Pr gallate	4·5	3′,4′,5′-Trihydroxypolyporic acid	4·5
Atromentin	3·5	3′,4′,5′-Trihydroxy-4″-t-butylpolyporic acid	65
Leucoatromentin	4·0		

With ferrous phthalocyanine (5×10^{-6}M) as catalyst in an ethyl benzoate solution (0·2M) of linoleic acid 3′,4′,5′-trihydroxypolyporic acid showed a protection factor of about 300, ten times that obtained with propyl gallate under analogous conditions. In aqueous emulsions of methyl linoleate or linoleic acid none of the compounds tested showed an antioxidant efficiency significantly above that of propyl gallate; however, the most promising compound, 3′,4′,5′-trihydroxy-4″-t-butylpolyporic acid, has not yet been tested in emulsions.

The above method for synthesis of unsymmetrically substituted polyporic acids is limited by several factors. First, steric hindrance makes it apparently impossible to prepare polyporic acid with substituents in the 2′-,6′-, 2″-, or 6″-positions. The nature of the substituents that can be introduced at other positions is largely governed by the stability or ease of preparation of the substituted nitrosoacetanilides or diazonium salts.

Hydrolysis of the 2,5-dichloro- to the 2,5-dihydroxy-quinones gives high yields of the corresponding substituted polyporic acids. In the synthesis of 3′,4′,5′-trihydroxypolyporic acid it was found unnecessary to use the reductive demethylation–oxidation method [3c,4] because the corresponding trimethoxy-compounds were demethylated smoothly by hydrobromic–acetic acid.

The antioxidant activity of hydroxypolyporic acids is related to that of flavonols. The common feature is the phenyl-enol grouping. Polyporic acid is, like flavonol, a very weak antioxidant but its activity is greatly enhanced by hydroxy-substituents in the terminal rings. If one assumes that the antioxidant activity is based on abstraction of a phenolic hydrogen atom, then in unsubstituted flavonol or polyporic acid abstraction of a hydrogen atom from the hydroxyl group adjacent to >C=O could not lead to mesomeric radicals embracing canonical quinonoid structures. This would, however, be the case when the phenyl substituent carries an *o*- or *p*-hydroxyl group [cf. II ⟷ III, and



(IV) ⟷ (V)]. The antioxidant efficiency of atromentin is accordingly much greater than that of polyporic acid but it is further increased by additional 3- and 5-hydroxyl

[4] Kögl and Becker, *Annalen*, 1928, **465**, 211, 243.

groups, allowing the formation of additional semiquinones. In linoleic acid a comparison of the antioxidant efficiency of 3′,4′,5′-trihydroxypolyporic acid and its 4″-t-butyl derivative appears to indicate that the t-butyl group contributes to the stabilisation of the intermediate radical. Surprisingly a relatively low protection factor was obtained with the non-butylated 3′,4′,5′-trihydroxypolyporic acid in methyl linoleate. This could be due to very slow dissolution of the antioxidant, a problem which does not arise with the t-butyl compound, the solubility of which is fifteen times greater. It is feasible that there is a difference between linoleic acid and methyl linoleate in this respect but one should also consider the novel interpretation of antioxidant action recently proposed by Eyring and his co-workers; [5] this could account for an effect of the polarity of the environment on the rate of antioxidant action.

Experimental

Embelin (2,5-*Dihydroxy*-3-*undecyl*-1,4-*benzoquinone*) *and* 2,5-*Dihydroxy*-3,6-*diundecyl*-1,4-*benzoquinone*.—These were prepared by Fieser and Chamberlin's methods.[6] The former, isolated from *Embelia ribes*, had m. p. 142—143°; the latter was synthesised by alkylation of 2,5-dihydroxy-1,4-benzoquinone with dilauroyl peroxide in acetic acid.

p-*t-Butylaniline*.—This was prepared by the Schmidt reaction from *p*-t-butylbenzoic acid (178 g.), with chloroform (300 ml.), sulphuric acid (500 ml.), and sodium azide (65 g.). The crude amine (85%), m. p. 12°, was acetylated (84% yield).

N-*Nitrosoacetanilide*.—Acetanilide in acetic acid was nitrosated with nitrogen dioxide. The nitrosoanilide [3b] (60%) had m. p. 53—54° (decomp.). *p*-Methoxy-*N*-nitrosoacetanilide [3b] (55%), m. p. 69—71° (decomp.), and *N*-nitroso-*p*-t-butylacetanilide [7] (73%), m. p. 60—61° (decomp.), were similarly prepared.

2,5-*Dichloro*-3-*phenyl*-1,4-*benzoquinone and* 2,5-*Dichloro*-3,6-*diphenyl*-1,4-*benzoquinone*.—Akagi's method [3c] was modified.* *N*-Nitrosoacetanilide was added to 2,5-dichlorobenzoquinone in acetone. The products were precipitated with water. Tar was extracted with 1 : 1 ethanol–ethyl acetate, and dichlorobenzoquinone was recovered by steam-distillation. The diaryl-quinone (18%), m. p. 214—215°, was fractionally crystallised (yellow needles) from dioxan, and the monoaryl-quinone (14%), m. p. 127—130°, was recrystallised (yellow needles) from ethanol.

The following were prepared by similar methods: 2,5-dichloro-3-*p*-methoxyphenyl-1,4-benzoquinone [3e] (22%), m. p. 135—137°, red prisms from ethanol–ethyl acetate; 2,5-dichloro-3,6-di-*p*-methoxyphenyl-1,4-benzoquinone [3c,d] (16%), m. p. 258—259°, brown needles from dioxan; 2,5-*dichloro*-3-p-*t-butylphenyl*- and 2,5-*dichloro*-3,6-*di*-p-*t-butylphenyl*-1,4-*benzoquinone* were separated by fractional crystallisation from ethanol, the former (31%) as orange needles, m. p. 141—143° (Found: C, 61·65; H, 4·6; Cl, 23·55. $C_{16}H_{14}Cl_2O_2$ requires C, 62·15; H, 4·55; Cl, 23·7%); the latter (11%) crystallised from toluene in orange needles, m. p. 319° (decomp.) (Found: C, 70·65; H, 5·75; Cl, 15·7. $C_{26}H_{26}Cl_2O_2$ requires C, 70·75; H, 5·95; Cl, 16·05%).

2,5-*Dichloro*-3-(3,4-*dimethoxyphenyl*)-6-p-*methoxyphenyl*-1,4-*benzoquinone*.—This was prepared (33%) as described by Akagi [3e] from 2,5-dichloro-3-*p*-methoxyphenyl-1,4-benzoquinone and diazotised 3,4-dimethoxyaniline, with ether–ethanol as solvent and aqueous sodium acetate as buffer. The diaryl-quinone, m. p. 229—232°, was a reddish-brown powder.

The following were similarly prepared: 2,5-*dichloro*-3-*phenyl*-6-(3,4,5-*trimethoxyphenyl*)-1,4-*benzoquinone* (20%) (from 2,5-dichloro-3-phenyl-1,4-benzoquinone and diazotised 3,4,5-trimethoxyaniline), pinkish-brown needles (from acetic acid), m. p. 234—235° (Found: C, 61·05; H, 3·8; Cl, 16·9. $C_{21}H_{16}Cl_2O_5$ requires C, 60·15; H, 3·85; Cl, 16·9%); and 2,5-*dichloro*-3-p-*t-butylphenyl*-6-(3,4,5-*trimethoxyphenyl*)-1,4-*benzoquinone* (28%) (from 2,5-dichloro-3-*p*-t-butylphenyl-1,4-benzoquinone and diazotised 3,4,5-trimethoxyaniline), dimorphic, (*a*) brown needles or (*b*) red prisms (both from toluene), m. p. 261° with transition of (*a*) to (*b*) at ~245° (Found: C, 63·05; H, 4·95; Cl, 15·0. $C_{25}H_{24}Cl_2O_5$ requires C, 63·15; H, 5·1; Cl, 14·9%).

* Since this work was completed, Cain has described a method which gives an improved yield of the monophenylquinone (*J.*, 1961, 936).

[5] Fueno, Ree, and Eyring, *J. Phys. Chem.*, 1959, **63**, 1940.
[6] Fieser and Chamberlin, *J. Amer. Chem. Soc.*, 1948, **70**, 71.
[7] Cadogan, Hey and Williams, *J.*, 1954, 3352.

Polyporic Acid.—This compound was prepared [3c] (56%) by hydrolysis of 2,5-dichloro-3,6-diphenyl-1,4-benzoquinone with potassium hydroxide in aqueous ethanol at 20° followed by acidification of the solution. Crystallisation from nitrobenzene gave brown flakes, m. p. 316—318°. The following symmetrical dihydroxy-quinones were similarly prepared: 4',4''-dimethoxypolyporic acid [3c] (80%; reaction at 35—40°), greenish-brown crystals with metallic lustre (from toluene), m. p. 294—295°; *4',4''-di-t-butylpolyporic acid* (62%; reaction at 55—60°), red flakes (from acetic acid), m. p. 306° (Found: C, 77·2; H, 6·8. $C_{26}H_{28}O_4$ requires C, 77·2; H, 7·0%) [*diacetate* (from acetic acid), orange flakes, m. p. 260—264° (decomp.) (Found: C, 73·8; H, 6·4. $C_{30}H_{32}O_6$ requires C, 73·75; H, 6·6%)]. A slightly different method [3e] was used for the preparation of the following dihydroxy-quinones, the potassium salts of which, being sparingly soluble in aqueous ethanol, were filtered off and dissolved in water before acidification: 3',4',4''-trimethoxypolyporic acid (88%), a reddish-brown powder, m. p. 258—259°; *3',4',5'-trimethoxypolyporic acid* (82%), pink crystals (from acetic acid), m. p. 208° (Found: C, 65·7; H, 4·8. $C_{21}H_{18}O_7$ requires C, 66·0; H, 4·75%) [*diacetate* (from ethanol), orange needles, m. p. 148—150° (decomp.) (Found: C, 64·3; H, 4·7. $C_{25}H_{22}O_9$ requires C, 64·35; H, 4·75%)]; *3',4',5'-trimethoxy-4''-t-butylpolyporic acid* (77%), brown-black dichroic needles (from ethanol), m. p. 216·5° (Found: C, 68·25; H, 5·85. $C_{25}H_{26}O_7$ requires C, 68·45; H, 6·0%) [*diacetate* (from ethanol), red flakes, m. p. 198—200° (Found: C, 66·7; H, 5·45. $C_{29}H_{30}O_9$ requires C, 66·65; H, 5·8%)].

3',4',5'-Trihydroxypolyporic Acid.—Hydrobromic acid (4·5 ml.; *d* 1·48) was added dropwise to a refluxing 4% solution of 3',4',5'-trimethoxypolyporic acid in acetic acid (17·5 ml.). The mixture was boiled for 3 hr. (solid began to separate after 1¾ hr.), cooled to 0°, and then filtered. After the precipitate had been washed with acetic acid and then with water, it was dried. The *product* (70%) crystallised (from nitrobenzene) in purplish-brown flakes, m. p. 295—303° (decomp.) (Found: C, 63·7; H, 3·65. $C_{21}H_{12}O_7$ requires C, 63·55; H, 3·55%). The yellow *penta-acetate*, crystallised from ethanol, had m. p. 200° (decomp.) (Found: C, 61·1; H, 4·2. $C_{28}H_{22}O_{12}$ requires C, 61·1; H, 4·05%). The trimethyl ether (prepared by gradual addition of 2·5N-sodium hydroxide to a refluxing solution of the hydroxy-quinone and dimethyl sulphate in ethanol) crystallised (from toluene) in brown-black needles, m. p. 208°, undepressed on admixture with 3',4',5'-trimethoxypolyporic acid.

The following compounds were demethylated by a similar method, except that the concentrations of starting materials were changed and the products were precipitated by the addition of water: 3',4',5'-trimethoxy-4''-t-butylpolyporic acid (7% solution in acetic acid) gave 3',4',5'-trihydroxy-4''-t-butylpolyporic acid which crystallised (from nitromethane) as dark brown needles, m. p. 240—250° (decomp.) (Found: C, 63·13, 62·75, 64·01, 64·14; H, 5·15, 4·84, 5·48, 5·41. $C_{22}H_{20}O_7$ requires C, 66·7; H, 5·1%) [*penta-acetate* (from 2-methoxyethanol), yellow rosettes, m. p. 291—221° (Found: C, 63·05; H, 5·10. $C_{32}H_{30}O_{12}$ requires C, 63·35; H, 5·0%)]. 3',4',4''-Trimethoxypolyporic acid (700 mg.; 1% solution in acetic acid) gave on demethylation a brown powder (556 mg., microscopic, doubly refracting, ovoid granules) which was almost insoluble in acetic acid and was unchanged by repeated crystallisation from nitrobenzene; this substance, which was expected to be identical with " leucomelone " (3',4',4''-trihydroxypolyporic acid [3a,e]) crystallised from 2-methoxyethanol–nitrobenzene as veined leaf-like crystals, m. p. >300° (decomp.) (Found: C, 63·0; H, 3·50; OMe, 0. Calc. for $C_{18}H_{12}O_7$: C, 63·55; H, 3·55%); with aqueous pyridine it gave a blue colour resembling that obtained with the other dihydroxy-quinones described; methylation, as described for 3',4',5'-trihydroxypolyporic acid, gave only starting material; the acetate (yellow prisms, m. p. 196—200°) decomposed on recrystallisation (from ethanol) and was very soluble in acetic acid.

Atromentin (4',4''-Dihydroxypolyporic Acid).—Fresh fruit-bodies of *Paxillus atrotomentosus*, in small pieces, were extracted with cold acetone (purple extract). The solvent was evaporated to a small volume at reduced pressure. Crude atromentin was filtered off, washed with water, dried, washed with light petroleum (b. p. 60—80°), dried (3·6% of dry weight of fungus), and crystallised from acetic acid. A portion was methylated (dimethyl sulphate–potassium hydroxide–aqueous 2-methoxyethanol), giving 4',4''-dimethoxypolyporic acid, which crystallised (from toluene) as greenish-brown flakes, m. p. 288° (cf. the synthetic compound above).

Atromentin was also prepared as described by Kögl and Becker [4] from 4',4''-dimethoxypolyporic acid by reductive demethylation (hydriodic acid–acetic acid), followed by atmospheric oxidation in 0·1N-sodium hydroxide. Attempts to prepare " leucomelone " from 3',4',4''-trimethoxypolyporic acid by a similar method [3e] were not successful. The product of reductive

demethylation appeared similar to that obtained by Akagi, but atmospheric oxidation in 0·1N- or N-sodium hydroxide gave only an amorphous product which did not crystallise from acetic acid or give the expected acetate. Other oxidising agents, *e.g.*, hydrogen peroxide, benzoquinone in dioxan, were tried without success.

Determination of Antioxidant Efficiency.—The course of autoxidation was followed by measuring oxygen uptake with conventional Warburg apparatus. Peroxide determination was carried out by the improved iodometric method described by Heaton and Uri.[8] In general, oxygen uptake was followed in duplicate experiments and the results compared with the iodometric peroxide estimation. The experiment was then repeated to check reproducibility. Oxygen uptake and peroxide were found to be equivalent, except in the case of the ferrous phthalocyanine-catalysed reaction where peroxide corresponded to only half the equivalent oxygen uptake, indicating peroxide decomposition. A more detailed description of this catalytic system will be given elsewhere.

The antioxidant was introduced in the following manner: 0·2 ml. of an M/5000-solution of the antioxidant in ethanol was pipetted into the central well of the Warburg flask; the flasks were then placed in a vacuum-desiccator, and the solvent was evaporated to dryness at ~1 mm.; 0·2 ml. of linoleic acid or methyl linoleate was introduced into the central well, the flask was immediately connected to the manometer, and the antioxidant was allowed to dissolve during continuous shaking of the flask in the Warburg bath. In the experiments in which ethyl benzoate was used as ultimate solvent the antioxidant solution was first evaporated to dryness in the body of the flask, then 3·5 ml. of the catalyst solution in ethyl benzoate were added, and the antioxidant was dissolved. Linoleic acid (0·2 ml.) was placed in the side-arm and washed into the body of the flask at zero time. A similar procedure was used for emulsions based on acetate buffers (of various pH) and Tween-80 or Triton X-100 as emulsifiers.

Solubility Data.—Solubilities of some of the new antioxidants in methyl oleate (25°), estimated spectrophotometrically, were: embelin, 5×10^{-4}M; 2,5-dihydroxy-3,6-diundecyl-1,4-benzoquinone, $2{\cdot}5 \times 10^{-3}$M; 3′,4′,5′-trihydroxypolyporic acid, $5{\cdot}1 \times 10^{-4}$M; 3′,4′,5′-trihydroxy-4″-t-butylpolyporic acid, $7{\cdot}6 \times 10^{-3}$M.

Linoleic Acid and Methyl Linoleate.—These were usually obtained from the Hormel Institute (Austin, Minnesota), but some experiments were carried out with samples prepared according to Heaton and Uri's directions.[9]

Thanks are given to Miss B. G. Allan and Miss J. Vassie for their assistance in the experimental work. The work was supported by U.S. Department of Agriculture PL-480 counterpart funds.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD, RESEARCH ESTABLISHMENT,
GREYHOPE ROAD, ABERDEEN.
[Present addresses: (G. J. B.) WAR OFFICE, C.D.E.E., PORTON DOWN,
SALISBURY, WILTS.
(N. U.) MINISTRY OF AVIATION, E.R.D.E.,
WALTHAM ABBEY, ESSEX.]

[*Received, August 28th*, 1961.]

[8] Heaton and Uri, *J. Sci. Food Agric.*, 1958, **9**, 781.
[9] Heaton and Uri, *J. Lipid Res.*, 1961, **2**, 152.