193. *Potential Anti-tumour Agents. Part I. Polyporic Acid Series.*

By B. F. Cain.

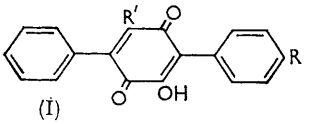
A series of compounds related to polyporic acid has been prepared for evaluation of their tumour-damaging capacity. Modifications not possessing the central 2,5-dihydroxyquinone nucleus have no anti-tumour activity.

Early in 1955 a screening programme was initiated for examining the indigenous New Zealand flora for anti-tumour agents. The first reproducible and significant results were obtained with a crude extract from the lichen *Sticta coronata* Mull Arg., whose biological activity, as indicated in a preliminary communication,[1] is due to the presence of polyporic acid (I; R = H, R′ = OH). The occurrence of this acid in *Sticta* species had been demonstrated previously by Murray,[2] but no prior reference to its anti-tumour effectiveness has been found although its anti-bacterial properties have been listed.[3]

In common with similar materials, to be described later, polyporic acid separates from large volumes of toluene in bronze plates of marked metallic appearance. However,

[1] Burton and Cain, *Nature*, 1959, **184**, 1326.
[2] Murray, *J.*, 1952, 1345.
[3] Akagi, Hirose, Watanabe, and Ose, *Ann. Proc. Gifu Coll. Pharm.* No. 4, 1954, **35**; *Chem. Abs.*, 1956, **50**, 14,865.

crystallization from dioxan or pyridine yields yellow or red solvates, respectively. The pyridine complexes should not be confused with the pyridinium salts. The anions of these materials (Table 2) are permanganate-coloured; addition of water to the red pyridine complexes yields such coloured solutions. Long drying of the solvates at elevated temperatures *in vacuo* is necessary to remove the solvents; in some cases even heating at 140° *in vacuo* was ineffective.



(I)

In an attempt to find the features of the molecule responsible for anti-tumour activity a series of derivatives has been prepared where one hydroxyl group has been replaced by H, SMe, OMe, SH, Me, and Cl.

Thiele acetylation of 2,5-diphenyl-1,4-benzoquinone with perchloric acid as catalyst proceeded normally, provided the temperature of the reaction was controlled. Hydrolysis, followed by mild oxidation, of the resultant hydroxyquinol triacetate yielded the monohydroxyquinone (I; R = R′ = H).

Further reaction of this material with methanethiol gave the methylthio-derivative (I; R = H, R′ = SMe) directly. Addition of mercaptoacetic acid gave a recalcitrant product and it was necessary to purify the material through the leucoacetate. The free mercaptoquinone (I; R = H, R′ = SH) was extremely unstable and attempts to crystallize it led to decomposition. Alkylation of the monohydroxyquinone (I; R = R′ = H) with acetyl peroxide [4] furnished the hydroxymethylquinone (I; R = H, R′ = Me) in excellent yield. The other variants listed above were best prepared by metathesis from 2,5-dichloro-3,6-diphenylquinone (see Experimental section).

All the compounds prepared that lacked the central 2,5-dihydroxy-groups were inactive against the L1210 leukemia in mice and had negligible antibacterial activity. Also, simpler molecules such as 2,5-dihydroxyquinone and 2,5-dihydroxy-3-phenyl-1,4-benzoquinone proved inactive.

The necessity of retaining the dihydroxyquinone grouping suggests that biological efficacy could be dependent on one or more of the following properties: the chelating power, the redox potential, or the acidity of the α-hydroxyquinone system. Quantitatively the three properties are inter-related and depend on the electron-density over the quinone ring. Accordingly, a series of polyporic acid derivatives with electron-donating and -attracting substituents on one phenyl group has been prepared.

Of numerous compounds prepared by arylating quinones with diazonium salts,[5] a few have been examined for their antibacterial properties.[6] A method of preparing 2,5-dichloro-3-phenyl-1,4-benzoquinone, in acceptable yield on a reasonable scale, from benzene diazo-acetate and 2,5-dichlorobenzoquinone is given in the Experimental section. Coupling of this with substituted arenediazonium salts yields the diaryldichloroquinones (see Table 1) that are readily hydrolyzed in high yield to the dihydroxyquinones (Table 2). This route is very flexible, and the only limitation is the requirement of a diazotizable amine.

The second arylation consistently gave poor yields (0—35%) that were not increased by the methods listed by Fieser *et al.*[4] The yields depend on the substitution pattern of the amines (*para* > *meta* ⩾ *ortho*) but probably more on the solubility of the products than on steric and electronic factors.

Most of these diaryldihydroxyquinones have some anti-tumour activity but no correlation between structure and biological activity is obvious.

[4] Fieser *et al.*, *J. Amer. Chem. Soc.*, 1942, **64**, 2060; 1948, **70**, 3151; Kvalnes, *Annalen*, 1934, **514**, 157; *J. Amer. Chem. Soc.*, 1934, **56**, 2478; 1948, **70**, 3201.

[5] Asano and Kameda, *J. Pharm. Soc. Japan*, 1939, **59**, 291.

[6] Asano and Huziwara, *J. Pharm. Soc. Japan*, 1939, **59**, 284.

Experimental

Extraction of Sticta coronata.—This was carried out essentially as described by Murray [2] and gave calycin (2·95%), m. p. 243—244° (lit.,[2,6] 242—244°), pulvinic anhydride (0·63%), m. p. 225—226° (lit.,[2,7] m. p. 222—224°, and polyporic acid (1·4%), m. p. 305° (sublimes). Polyporic acid was compared directly with a synthetic specimen.[8]

2′,3′,5′-Triacetoxy-p-*terphenyl*.—To a suspension of 2,5-diphenyl-1,4-benzoquinone (10 g.) in acetic anhydride (65 ml.) 72% perchloric acid (1 ml.) was added dropwise with stirring. The suspension was kept at 55° until a homogeneous solution resulted. After a further 4 hr. at room temperature the excess of anhydride was decomposed with water, and the *triacetate* collected, m. p. 189—191° (9·6 g.). Repeated crystallization from methanol gave prisms, m. p. 192—193° (Found: C, 71·5; H, 4·9. $C_{24}H_{20}O_6$ requires C, 71·3; H, 5·0%).

3-Hydroxy-2,5-diphenyl-1,4-benzoquinone.—Sodium (1·8 g.) was added to a suspension of the above triacetate (10 g.) in absolute methanol (50 ml.), and the suspension stirred till homogeneous. Addition to a solution of ferric chloride hexahydrate (13·5 g.) in acetic acid (25 ml.) and concentrated hydrochloric acid (15 ml.) gave a deep red solution which deposited the *quinone* (6·3 g.) on addition of water (50 ml.). Crystallization from acetic acid gave scarlet prisms, m. p. 226—227° (Found: C, 78·1; H, 4·1. $C_{18}H_{12}O_3$ requires C, 78·2; H, 4·4%).

2-Hydroxy-5-methylthio-3,6-diphenyl-1,4-benzoquinone.—A solution of the hydroxyquinone (2 g.) in ethanol (1 l.) was treated with a ten-fold excess of methanethiol overnight. 2 Equivs. of ferric chloride in ethanol was then added, followed by concentrated hydrochloric acid (2 ml.). Dilution with water (2 l.) after a further 2 hr. precipitated the *methylthio-quinone*. Crystallization from small volumes of acetic acid gave vermilion needles (1·36 g.), m. p. 151—153° (Found: C, 70·9; H, 4·3; S, 10·1. $C_{19}H_{14}O_3S$ requires C, 71·1; H, 4·4; S, 10·0%).

2-Hydroxy-5-mercapto-3,6-diphenyl-1,4-benzoquinone.—Use of mercaptoacetic acid in place of methanethiol in the preceding experiment gave a crude product (2·1 g.) which was suspended in acetic anhydride (10 ml.) and refluxed with zinc dust (3 g.) for 10 min. Isolation in the usual manner gave a *tetra-acetate*, needles (from ethanol), m. p. 243—244° (Found: C, 65·4; H, 4·5; S, 6·4. $C_{26}H_{22}O_7S$ requires C, 65·1; H, 4·6; S, 6·7%). To a suspension of this (1·6 g.) in methanol (10 ml.), sodium (0·5 g.) was added and the solution was stirred at room temperature in air for 0·5 hr., then filtered and slowly acidified in the cold with just sufficient methanolic hydrogen chloride. The crimson precipitate was washed by suspension in water, collected, and stored *in vacuo*. Attempted recrystallization led to a dark brown insoluble material, presumably the disulphide since reductive acetylation re-formed the leucoacetate recorded above. On being heated, the *mercapto-quinone* gradually darkened but did not melt below 360° (Found: C, 70·0; H, 3·7; S, 10·1. $C_{18}H_{12}O_3S$ requires C, 70·1; H, 3·9; S, 10·4%).

2-Hydroxy-5-methyl-3,6-diphenyl-1,4-benzoquinone.—An acetic acid solution of 3-hydroxy-2,5-diphenylbenzoquinone (1·46 g.) was kept at 90—95° while freshly prepared ethereal acetyl peroxide (0·62 g. of peroxide) was introduced beneath the surface. Heating at 95° was continued until gas evolution ceased. Evaporation and crystallization from aqueous dioxan gave the *methyl-quinone* as orange-red needles (1·22 g.), m. p. 154—155° (Found: C, 78·3; H, 5·1. $C_{19}H_{14}O_3$ requires C, 78·6; H, 4·9%).

2,5-Dimethyl-3,6-diphenyl-1,4-benzoquinone.—This *compound* was prepared by using 2,5-diphenylbenzoquinone (2 g.) and 2 mol. of acetyl peroxide. It separated from aqueous dioxan in lemon-coloured needles, m. p. 146—147° (Found: C, 83·1; H, 5·5. $C_{20}H_{16}O_2$ requires C, 83·3; H, 5·6%).

2,5-Dichloro-1,4-benzoquinone.—A suspension of quinol (500 g.) in concentrated hydrochloric acid (2·5 l.) was stirred rapidly while powdered potassium chlorate (379 g.) was added portionwise so that the temperature remained between 40° and 45°. After 12 hr. at 0° the dichloroquinol was collected and immediately oxidized with sodium dichromate in aqueous sulphuric acid. The dichloro-quinone (476 g.) separated from ethanol in orange prisms, m. p. 158—160°.

2,5-Dichloro-3-phenyl-1,4-benzoquinone.—Aniline (65·7 ml.) in water (100 ml.) and concentrated hydrochloric acid (216 ml.) was diazotised with sodium nitrite (54·6 g.) in water (150 ml.), then added to a solution of 2,5-dichlorobenzoquinone (105·5 g.) in methanol (3·3 l.) and ether (900 ml.) with stirring while anhydrous sodium acetate (120 g.) in water (200 ml.) was added

[7] Hesse, *J. prakt. Chem.*, 1900, **62**, 334.
[8] Shildmeck and Adams, *J. Amer. Chem. Soc.*, 1931, **53**, 2373.

at an equivalent rate, the temperature being kept below 40°, 100 ml. of water being used for rinsing. The solution was stirred for a further 2 hr., then solvent (1680 ml.) was removed by distillation through a short fractionating column. Hot water (900 ml.) was added and the boiling solution filtered rapidly. The required *quinone* separated on cooling. Crystallization from ethanol (charcoal) gave material (62 g.), m. p. 121—124°, suitable for further coupling. Repeated crystallization gave pure material as lemon-coloured plates, m. p. 127—128° (Found: C, 56·9; H, 2·4; Cl, 28·4. $C_{12}H_6Cl_2O_2$ requires C, 56·9; H, 2·4; Cl, 28·0%).

TABLE 1. 2,5-*Dichloro*-3-p-R-*phenyl*-6-*phenyl*-1,4-*benzoquinones*.

			Found (%)					Required (%)			
R	Solvent	M. p.	C	H	Cl	N	Formula	C	H	Cl	N
H	Bu^{n}OH	209—210° [a]	66·0	3·2	21·2	—	$C_{18}H_{10}Cl_2O_2$	65·7	3·1	21·0	—
OMe	EtOH	184—186 [b]	63·3	3·0	19·3	—	$C_{19}H_{12}Cl_2O_3$	63·5	3·1	19·7	—
Me	AcOH	212—213 [c]	66·4	3·6	20·7	—	$C_{19}H_{12}Cl_2O_2$	66·5	3·5	20·7	—
Cl	Bu^{n}OH	234—235	59·1	2·4	29·1	—	$C_{18}H_8Cl_4O_2$	59·4	2·5	29·3	—
SMe	EtOH	228—229	60·6	3·1	18·7	—	$C_{19}H_{12}Cl_2O_2S$	60·8	3·2	18·9	—
NHAc	AcOH	229—300	62·0	3·2	—	3·4	$C_{20}H_{13}Cl_2NO_3$	62·2	3·4	—	3·6
NMeAc	EtOH	245—246	63·0	3·8	—	3·5	$C_{21}H_{15}Cl_2NO_3$	63·0	3·8	—	3·5
NMe_2	AcOH–EtOH	193—194	64·6	4·2	—	3·7	$C_{20}H_{15}Cl_2NO_2$	64·5	4·1	—	3·7
$CO{\cdot}NH_2$	EtOH	283—284	61·0	3·0	—	3·3	$C_{19}H_{11}Cl_2NO_3$	61·3	3·0	—	3·8
$SO_2{\cdot}NH_2$	AcOH	284 [d]	52·9	2·9	—	3·1	$C_{18}H_{11}Cl_2NO_4S$	52·9	2·7	—	3·4
$SO_2{\cdot}Me$	EtOH	267—268	56·2	3·1	17·6	—	$C_{19}H_{12}Cl_2SO_4$	56·0	3·0	17·4	—
CO_2Me	MeOH	229—230	61·9	3·2	17·9	—	$C_{20}H_{12}Cl_2O_4$	62·0	3·1	18·3	—

[a] Kvalnes [4] gives m. p. 207—208°. [b] Lit.,[5] 183—185°. [c] Lit.,[5] 204—205°. [d] With decomp.

TABLE 2. *Polyporic acid derivatives* (I; R′ = OH).

			Found (%)				Required (%)		
R	Solvent	M. p.	C	H	N	Formula	C	H	N
H [a]	Toluene	305° (subl)	74·2	4·3	—	$C_{18}H_{12}O_4$	74·0	4·1	—
OMe	Pyridine	267—269	70·5	4·3	—	$C_{19}H_{14}O_5$	70·8	4·4	—
Me	Pyridine	259—260 [b]	74·3	4·5	—	$C_{19}H_{14}O_4$	74·5	4·6	—
Cl	Toluene	289—291	66·3	3·5	—	$C_{18}H_{11}ClO_4$	66·2	3·4	—
SMe	Pyridine	285—287	67·3	4·1	—	$C_{19}H_{14}O_4S$ [d]	67·5	4·2	—
NHAc	Pyridine	307—309	68·5	4·5	3·7	$C_{19}H_{13}NO_5$	68·8	4·3	4·0
NMeAc	Pyridine–ligroin	226—228	69·0	5·1	3·8	$C_{21}H_{17}NO_5$	69·4	4·7	3·9
NMe_2	Pyridine	241 [e]	71·4	5·0	4·1	$C_{20}H_{17}NO_4$	71·6	5·1	4·2
$CO{\cdot}NH_2$	Pyridine	>360	67·9	4·0	4·2	$C_{19}H_{13}NO_5$	68·1	3·9	4·2
$SO_2{\cdot}NH_2$	Pyridine	>360	61·1	3·9	6·5	$C_{23}H_{18}N_2O_6S$	61·3	4·0	6·2
$SO_2{\cdot}Me$	Bu^{n}OH	192—193	61·2	3·4	—	$C_{19}H_{14}O_6S$ [f]	61·6	3·7	—
CO_2Me	Dioxan–H_2O	324—328	68·5	4·2	—	$C_{20}H_{14}O_6$	68·6	4·0	—

[a] Polyporic acid. [b] Lit.,[5] 254°. [c] With decomp. [d] Found: S, 9·7. Reqd.; S, 9·5%. [e] Includes one mol. of pyridine of crystallization, not removed on drying *in vacuo*. [f] Found: S, 9·0. Reqd.: S, 8·7%.

The by-product remaining from the filtration was 2,5-dichloro-3,6-diphenyl-1,4-benzoquinone which crystallized from butan-1-ol as orange-yellow plates (11·1 g.), m. p. 209—210° (Kvalnes [4] gives m. p. 207—208°) (Found: C, 66·0; H, 3·2; Cl, 21·3. Calc. for $C_{18}H_{10}O_2Cl_2$: C, 65·7; H, 3·1; Cl, 21·0%).

2,5-*Diaryl*-3,6-*dichlorobenzoquinones*.—To a solution of the dichlorophenylbenzoquinone (5 g.) in ethanol (150 ml.) was added alternately a solution of the diazonium salt from 1·2 mol. of amine (prepared in the minimum volume of water) and a saturated solution of sufficient sodium acetate to neutralize the excess of acid. Alternatively an acetylnitrosoamine may be used instead of the diazotized amine. After 12 hr. the solution was heated to the b. p. and water added to turbidity. The crystals, oil, or tar obtained on refrigeration was dealt with according to its nature.

Hydrolysis of Dichloroquinones to Dihydroxyquinones.—To the pure dichloroquinone was added sufficient methanol to wet the crystals, followed by 10% aqueous sodium hydroxide (8 equiv.), and the suspension stirred until a solution resulted, for which occasionally mild heating was required. After dilution with an equal volume of water the solution was filtered and strongly acidified, and the *hydroxyquinone* collected and crystallized (see Table 2). The m. p. depend on the rate of heating and are not always sharp. The figures quoted were obtained with heating at 2°/min. from 20° below the m. p.

2-*Chloro*-5-*methoxy*-3,6-*diphenyl*-1,4-*benzoquinone*.—Sodium methoxide (1 mol.) in methanol was added with stirring to a solution of dichlorodiphenylbenzoquinone (2 g.) in methanol. Evaporation to incipient separation and cooling gave the *product* (1·6 g.), orange-red prisms (from di-n-pentyl ether), m. p. 180—181° (Found: C, 70·8; H, 4·1; Cl, 10·4. $C_{19}H_{13}O_3Cl$ requires C, 70·3; H, 4·0; Cl, 10·9%).

2-*Hydroxy*-5-*methoxy*-3,6-*diphenyl*-1,4-*benzoquinone*.—To the chloromethoxyquinone (2 g.) in aqueous dioxan was added 10% aqueous sodium hydroxide (2 mol.), and the mixture heated on the water-bath for 1 hr., diluted with an equal volume of water, filtered, and acidified. Crystallization from aqueous dioxan gave the *hydroxyquinone* as chocolate-brown needles (1·36 g.), m. p. 210—211° (Found: C, 74·3; H, 4·8. $C_{19}H_{14}O_4$ requires C, 74·5; H, 4·6%).

2,5-*Dimethoxy*-3,6-*diphenyl*-1,4-*benzoquinone*.—This *compound*, prepared from the corresponding dichloroquinone by the action of 2 mols. of methanolic sodium methoxide in quantitative yield, crystallized from acetic acid, in red, diamond-shaped plates, m. p. 202—203° (Found: C, 75·9; H, 4·8; OMe, 18·9. $C_{20}H_{16}O_4$ requires C, 75·0; H, 5·0; 2OMe, 19·4%).

2-*Chloro*-5-*hydroxy*-3,6-*diphenyl*-1,4-*benzoquinone*.—Solutions of the dichlorodiphenylquinone (2 g.) in dioxan, and potassium hydrogen carbonate (0·6 g.) in water, were mixed and heated for 4 hr. on the water-bath; the whole was filtered after addition of an equal volume of water and then acidified. The *product* separated from dioxan in yellow needles (1·28 g.), m. p. 211—212° (Found: C, 70·0; H, 3·4; Cl, 11·4. $C_{18}H_{11}O_3Cl$ requires C, 69·6; H, 3·6; Cl, 11·4%).

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Cancer Research Laboratory, Cornwall Hospital,
Auckland, S.E.4, New Zealand.

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