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# ARYL-2-HALOGENOALKYLAMINES—XXIV.
## DERIVATIVES OF *o*-, *m*- AND *p*-AMINOPHENOL: SYNTHESIS AND ANTINEOPLASTIC ACTIVITIES

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**Abstract**—The preparation of *o*-, *m*- and *p*-benzyloxy- and *o*- and *p*-hydroxy-(*NN*-di-2-chloroethyl)aniline is described. The results of a preliminary screening against the transplanted Walker rat carcinoma and the mouse lymphoid leukaemia, L1210, are reported.

IN VIEW of the suggestion that the high activity of *NN*-di-2-chloroethylamine against the ADJ/PC5 plasma cell tumour might be connected with its conversion, *in vivo*, into the *p*-hydroxy derivative[1] it became of interest to prepare other hydroxylated derivatives and to compare their carcinostatic activities.

### MATERIALS

*p*-(Di-2-chloroethylamino)phenol was originally prepared in low yield by the action of phosphoryl chloride on *p*-(di-2-hydroxyethylamino) phenol, obtained by reacting ethylene oxide with *p*-aminophenol in dilute acetic acid solution.[2] Benn *et al.*[3] achieved improved yields by protecting the phenolic group as the benzyl ether during the treatment with phosphoryl chloride and then regenerating the phenol by hydrogenolysis. On attempting to apply this method to the preparation of the *o*-derivative difficulty was encountered because *o*-aminophenol does not react with ethylene oxide under the same conditions as the other isomers. The benzyl ether of *o*-aminophenol is readily hydroxyethylated[4] and it has been found convenient to use benzyl ethers as starting materials for the preparation of the three isomers.

The benzyloxyanilines had previously been obtained from the acetamidophenols but the overall yields were not high due to the vigorous conditions required to remove the acetyl groups. Improved yields were obtained if the amino group was protected during benzylation by the readily removed phthaloyl group.

The aminophenols were converted into the known phthalimidophenols which were then treated with benzyl bromide in ethanolic potassium ethoxide giving the benzyl phthalimidophenyl ethers (I). Conversion into the benzyloxyanilines (II) was achieved under mild conditions and in good yield by heating with hydrazine hydrate in ethanol. All three isomers readily reacted with ethylene oxide in aqueous acetic acid to give the (di-2-hydroxyethylamino)phenyl ethers (III, R = $PhCH_2$, X = OH) and these were converted into the (di-2-chloroethylamino)phenyl ethers (III, R = $PhCH_2$, X = Cl) by treatment with phosphoryl chloride. Hydrogenolysis over a palladium-charcoal catalyst afforded the free phenols (III, R = H, X = Cl) from

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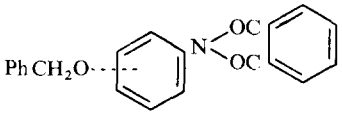
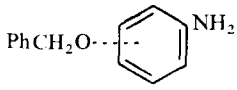
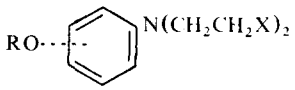
the *o*- and *p*-isomers. However in the case of the *m*-isomer unexpected cleavage of the nitrogen-phenyl bond occurred and this precluded the preparation of *m*-(di-2-chloroethylamino)phenol by this method.




(I)




(II)




(III)

Melting points were determined with a Gallenkamp heated metal block apparatus and are corrected. Infra red spectra were recorded on a Perkin–Elmer spectrophotometer Model 137B.

*Benzyl* o-*phthalimidophenyl ether*

*o*-Phthalimidophenol[5] (23·9 g) was added to a solution of potassium (3·9 g) in ethanol (250 ml). Benzyl bromide (15 ml) was added dropwise with vigorous stirring and the mixture was then heated for 1 hr on a steam bath. The oil, which separated on pouring the cooled mixture on to ice, soon solidified and was collected and washed with dilute sodium hydroxide and then with light petroleum (b.p. 30–40°). *Benzyl* o-*phthalimidophenyl ether* (23 g, 70%) formed prisms, m.p. 147–148°, from ethanol, $\nu_{max}^{nujol}$ 1700 $cm^{-1}$. (Found: C, 76·3%; H, 4·6%; N, 4·5%. Calc. for $C_{12}H_{15}NO_3$: C, 76·6%; H, 4·6%; N, 4·3%.)

*Benzyl* m-*phthalimidophenyl ether*. Needles, m.p. 128°, from ethanol, $\nu_{max}^{nujol}$ 1700 $cm^{-1}$, similarly prepared from *m*-phthalimidophenol[5] in 90% yield. (Found: C, 76·2%; H, 4·7%; N, 4·5%.)

*Benzyl* p-*phthalimidophenyl ether*. Needles, m.p. 238°, from dimethylformamide, $\nu_{max}^{nujol}$ 1700 $cm^{-1}$, obtained in 85% yield from *p*-phthalimidophenol.[6] (Found: C, 76·3%; H, 4·5%; N, 4·3%.)

o-*Benzyloxyaniline*

A suspension of benzyl *o*-phthalimidophenyl ether (10 g) in ethanol (100 ml) containing hydrazine hydrate (1·5 ml) was heated under reflux for 1 hr. The precipitate which formed on adding ether (300 ml) to the cooled solution was collected and the filtrate was evaporated under reduced pressure. The residue was extracted with ether (100 ml) and the oil obtained on evaporating the extract was heated under reflux with dilute hydrochloric acid (100 ml, 2N). On adding an excess of concentrated aqueous sodium hydroxide to the cooled mixture an oil separated and this was extracted with ether. The dried ($Na_2SO_4$) extract yielded *o*-benzyloxyaniline, 85%, b.p. 156–160°/0·5–1·0 mm, m.p. 37–39° (lit.[7] m.p. 38–39°), $\nu_{max}^{film}$ 3300, 3225, 740 and 698 $cm^{-1}$. *m*-Benzyloxyaniline, b.p. 162–166°/0·1 mm, m.p. 61–62° (lit.[8] m.p. 61–62·5°), $\nu_{max}^{nujol}$ 3350 and 3250 $cm^{-1}$ and *p*-benzyloxyaniline, m.p. 53–55° (lit.[9] m.p. 54–55°), $\nu_{max}^{nujol}$ 3220 and 3150 $cm^{-1}$, were similarly prepared in 80% yield.

*Benzyl* m-(*di-2-hydroxyethylamino*)*phenyl ether*

A solution of *m*-benzyloxyaniline (35 g) in glacial acetic acid (250 ml) and water (250 ml) containing ethylene oxide (70 ml) was kept at room temperature for 48 hr. After evaporation under reduced pressure, water (500 ml) was added and the oil which separated was extracted with chloroform. This extract was washed with saturated

aqueous $NaHCO_3$, then with water, and finally dried ($Na_2SO_4$). The oil (40 g), obtained on evaporation, which exhibited an intense absorption band at 3300 $cm^{-1}$, was shown to be benzyl *m*-(di-2-hydroxyethylamino)phenyl ether by the preparation of its *di*-(p-*nitrobenzoate*), yellow prisms, m.p. 131–133°, $\nu_{max}^{nujol}$ 1720 $cm^{-1}$, from acetone. (Found: C, 63·5%; H, 5·0%; N, 7·3%. Calc. for $C_{31}H_{27}N_3O_9$: C, 63·6%; H, 4·7%; N, 7·2%.)

*Benzyl* p-(*di-2-hydroxyethylamino*)*phenyl ether*. This was similarly prepared from *p*-benzyloxyaniline, it formed small plates, m.p. 91–93° (lit.[3] m.p. 93–94°), $\nu_{max}^{nujol}$ 3250 $cm^{-1}$.

*Benzyl* o-(*di-2-chloroethylamino*)*phenyl ether*

Benzyl *o*-(di-2-hydroxyethylamino)phenyl ether[4] (20 g) and phosphoryl chloride (20 ml) in benzene (100 ml) were allowed to stand at room temperature for 12 hr and then heated under reflux for 1 hr. After pouring the cooled mixture on to ice the benzene layer was separated, washed with saturated aqueous $NaHCO_3$, then with water, dried ($Na_2SO_4$), and evaporated under reduced pressure. The residual oil was purified by passing a benzene solution through a column of deactivated alumina (prepared by treating Spence Type H alumina (100 g) with dilute acetic acid (10 ml, 10%)). The product, which exhibited no OH absorption near 3300 $cm^{-1}$, was shown to be benzyl *o*-(di-2-chloroethylamino)phenyl ether by the preparation of its *hydrochloride*, prisms, m.p. 129–131°, $\nu_{max}^{nujol}$ 3200 and 2100 $cm^{-1}$, from benzene-light petroleum (b.p. 30–40°). (Found: C, 56·7%; H, 5·8%; Cl, 28·9%; N, 4·1%. Calc. for $C_{17}H_{20}Cl_3NO$: C, 56·6%; H, 5·6%; Cl, 29·3%; N, 3·9%) and its *picrate*, needles, m.p. 111–113°, from ethanol. (Found: C, 50·3%; H, 4·1%; Cl, 13·1%; N, 9·9%. Calc. for $C_{23}H_{22}Cl_2N_4O_8$: C, 49·9%; H, 4·0%; Cl, 12·8%; N, 10·1%.)

*Benzyl* m-(*di-2-chloroethylamino*)*phenyl ether*

Benzyl *m*-(di-2-hydroxyethylamino)phenyl ether, after purification by passing a chloroform solution through a column of deactivated alumina, was similarly converted into the di-2-chloroethyl derivative, which formed a *hydrochloride*, prisms, m.p. 118–120°, $\nu_{max}^{nujol}$ 3300 and 2280 $cm^{-1}$, from benzene-light petroleum (b.p. 30–40°). (Found: C, 56·7%; H, 5·5%; Cl, 29·4%; N, 4·0%.)

*Benzyl* p-(*di-2-chloroethylamino*)*phenyl ether*. M.p. 100–102° (lit.[3] m.p. 105–106°) was similarly obtained from the above di-2-hydroxyethyl derivative and converted into the phenol by hydrogenolysis.[3]

o-(*Di-2-chloroethylamino*)*phenol*

A suspension of benzyl *o*-(di-2-chloroethylamino)phenyl ether hydrochloride (5 g) in ethanol (200 ml) containing palladium-charcoal (0·5 g, 5% Pd) was shaken in an atmosphere of hydrogen for 1 hr when the theoretical amount of gas was taken up. After evaporating the filtered solution the residue solidified on covering with ether saturated with dry HCl gas. On crystallization from ethanol-ether containing HCl the *hydrochloride* of o-(*di-2-chloroethylamino*)*phenol* formed prisms (2·5 g), m.p. 134–135°, $\nu_{max}^{nujol}$ 3100–2400 $cm^{-1}$. The salt tends to lose HCl on keeping—this accounts for the somewhat high carbon and low chlorine figures for the dried analytical specimen. (Found: C, 45·0; H, 5·5; Cl, 38·6%; N, 5·2%. Calc. for $C_{10}H_{14}Cl_3NO$: C, 44·4%; H, 5·2%; Cl, 39·4%; N, 5·2%). After refluxing a freshly prepared specimen in

50% aqueous acetone for 7 hr titration of the neutralized solution with standard silver nitrate (dichlorofluorescein indicator) indicated a chlorine content of 39·1 per cent.

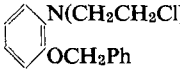
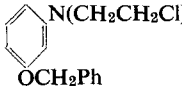
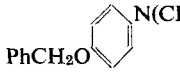
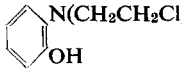
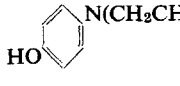
*Hydrogenation of benzyl* m-(*di-2-chloroethylamino*)*phenyl ether hydrochloride*

A suspension of the hydrochloride (5·5 g) in ethanol (200 ml) ethyl acetate (200 ml) containing palladium-charcoal (1 g, 5% Pd) was shaken in an atmosphere of hydrogen. The reaction was much slower than in the case of the *o*-isomer and hydrogen uptake only ceased after 10 hr (1·3 × theory). The residue, obtained after evaporating the filtered solution, was covered with light petroleum (b.p. 30–40°) and on standing crystals, m.p. 209–210°, separated. No depression of m.p. occurred on admixture with di-2-chloroethylamine hydrochloride (m.p. 210–211°) and the i.r. spectra of the two compounds were identical. The yield of di-2-chloroethylamine hydrochloride was 1 g (37 per cent of theory).

## METHODS

The protocol for testing the compounds as inhibitors of the growth of the transplanted Walker rat carcinoma 256 and the method of assay against the mouse lymphoid leukaemia, L1210, are described in Part XXII.[4] In both tests compounds were administered by a single i.p. injection in arachis oil on the day following implantation or inoculation. In Table 1 the results of the Walker tumour test are expressed as

TABLE 1. SCREENING AGAINST WALKER 256 (S.C.) TUMOUR

| Compound | % Reaction* H | % Reaction* Cl | Dose† mg/kg | Survivors | C/T† ratio | Approx. $LD_{50}$‡ mg/kg |
|---|---|---|---|---|---|---|
|  | 85 | 86 | 640<br>320<br>160<br>80<br>40 | 0/3<br>1/3<br>3/3<br>3/3<br>3/3 | —<br>∞<br>120<br>10<br>1·3 | 280 |
|  | 8 | 9 | 800<br>400<br>200<br>100<br>50 | 1/3<br>3/3<br>3/3<br>3/3<br>3/3 | 1<br>1·9<br>1<br>1<br>1 | 700 |
|  | 53 | 54 | 320<br>160<br>80<br>40<br>20 | 0/3<br>3/3<br>3/3<br>3/3<br>3/3 | —<br>∞<br>∞<br>10<br>1·6 | 240 |
|  | 79 | 80 | 32<br>16<br>8<br>4 | 0/3<br>3/3<br>3/3<br>3/3 | —<br>∞<br>13<br>1·3 | 24 |
|  | 56 | 56 | 8<br>4<br>2<br>1 | 0/3<br>3/3<br>3/3<br>3/3 | —<br>71<br>2<br>1 | 6 |

* Release of hydrogen or chloride ions on refluxing for ½ hr in 1:1 acetone-water $C = 0{\cdot}02$ M for chloroethylamines or 0·01 M for di(chloroethyl)amines (see ref. 10).

† See text.

‡ For tumour bearing rats.

C/T ratios, that is, the weight of the tumours in control rats/the weight in treated rats. The T/C ratio shown for the L1210 assay (Table 2) equals [the average survival time of treated mice/the average survival time of controls] × 100.

TABLE 2. SCREENING AGAINST MOUSE LEUKAEMIA, L1210

| Compound | Dose† mg/kg | T/C† ratio | $LD_{50}$ for host mouse mg/kg |
|---|---|---|---|
|  | 280<br>140<br>70 | 107<br>93<br>107 | 280 |
|  | 60<br>30<br>15 | 64<br>125<br>123 | 60 |
|  | 1600<br>800<br>400 | 96<br>106<br>104 | >1600 |

† See footnote to Table 1.

## RESULTS AND DISCUSSION

*Chemical reactivity*

The benzyloxy substituents have the expected effect on the chemical reactivity of the di-2-chloroethylamino group. Di-(2-chloroethyl)aniline reacts to the extent of 20 per cent under the standard conditions and this is increased to 54 per cent by the insertion of an electron releasing *p*-benzyloxy group and to 86 per cent by an *o*-benzyloxy substituent—there being an additional increase due to the effect of the bulky *o*-substituent on the coplanarity of the nitrogen valency bonds with the aromatic ring. In the *m*-position the benzyloxy substituent has an electron withdrawing effect and the chemical reactivity is reduced (compare the relative effects of *o*-, *m*- and *p*-methoxy groups)[11]. Hydroxy groups have practically the same effects on chemical reactivity as the ether groups since under the standard conditions the phenolic group will be unionized.

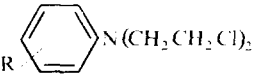
*Toxicities*

The *m*-benzyloxy derivative, which is considerably less reactive than the *o*- and *p*-isomers, is the least toxic. The *o*- and *p*-hydroxy derivatives of di(2-chloroethyl) aniline are more toxic than the corresponding benzyl ethers and the parent nitrogen mustard. The *p*-hydroxy derivative is significantly more toxic than the *o*-isomer although the chemical reactivity is somewhat lower.

*Antineoplastic activities*

*o*-Hydroxy- and *o*-benzyloxy-di-(2-chloroethyl)aniline are active inhibitors of the growth of the transplanted Walker tumour (Table 1) and although the potency differs by a factor of over ten the selectivity of action, as indicated by the chemotherapeutic index (Table 3), is similar. There is an even bigger increase in potency in the case of

TABLE 3.

| Compound  | Assay against Walker 256 tumour in Wistar rats | | | Assay against ADJ/PC5 tumour in BALB/C⁻ mice (ref. 1) | | |
|---|---|---|---|---|---|---|
| | $LD_{50}$ mg/kg | $ED_{90}$* mg/kg | C.I.† | $LD_{50}$ mg/kg | $ED_{90}$ mg/kg | C.I.† |
| R = *o*-$PhCH_2O$ | 280 | 80 | 3·5 | — | — | — |
| R = *m*-$PhCH_2O$ | 700 | inactive | — | — | — | — |
| R = *p*-$PhCH_2O$ | 240 | 40 | 6 | — | — | — |
| R = *o*-OH | 24 | 8 | 3 | — | — | — |
| R = *p*-OH | 6 | 3·6 | 1·7 | 29 | 19 | 1·5 |
| R = *p*-$CH_3O$ | 75 | 16·5 | 4·5 | 170 | 61 | 2·8 |
| R = H | 146 | 13·6 | 10·8 | 117 | 30 | 3·9 |

* Dose required to produce 90% inhibition of tumour growth.

† $LD_{50}/ED_{90}$.

the *p*-isomers but the ether shows the higher selectivity of action. The *m*-benzyloxy derivative of low chemical reactivity shows no anti-tumour activity. *o*-(Di-2-chloroethylamino)phenol exhibits moderate activity against the L1210 lymphoid leukemia (25 per cent increase in survival time, Table 2). Table 3 shows the chemotherapeutic index of the new compounds and of some related chloroethylarylamines when assayed against the Walker tumour and the ADJ/PC5 plasma cell tumour.[1] The unsubstituted aniline derivative shows a higher index in both tests than the *p*-hydroxy and *p*-methoxy derivatives.

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