The Antibacterial Activity of Simple Derivatives of 2-Aminophenol

By MARY BARBER, *British Postgraduate Medical School, London, W. 12*,
AND G. A. D. HASLEWOOD, *Guy's Hospital Medical School, London, S.E. 1*

(Received 30 April 1945)

Discovery of the activity of 2- and 4-aminophenol against several bacterial species, including Gram-negative bacilli, led us to investigate systematically the effect of nuclear substitution on the behaviour of these substances. Preliminary work (Barber & Haslewood, 1944) made us conclude that substituted 4-aminophenols, though possibly active, would be too unstable for therapeutic use, but that a series of 4-substituted 2-aminophenols of the general formula (I) ought to be further investigated. We have now prepared and examined members of this series from $R=H$ to $R=\text{butyl}$, and these compounds, with some simple relatives, are listed in Table 1, which also shows approximate antibacterial activities.

Antibacterial activity was measured *in vitro* by the methods already described (Barber & Haslewood, 1944). The results shown in Table 1 represent the limiting dilution of each compound causing

complete bacteriostasis in nutrient broth, when a small inoculum of less than 500 viable organisms was added.

We conclude that nuclear substitution of 2-aminophenol may provide substances of different antibacterial activity: however, none of the compounds tested by us was definitely more active than 2-aminophenol itself.

The preparation of the substituted 2-aminophenols was effected by hyposulphite reduction of the corresponding nitro-compounds. These were made from the 4-substituted phenols, which were in general the known reference substances in the series. Of these phenols, 4-*isobutyl* phenol does not appear to have been previously crystallized; Niederl, Niederl, Shapiro & McGreal (1937) described it as an oil, whereas our product had m.p. 51–52°.

We made 4-*isopropyl* phenol from cumene (Bert, 1923), but this method, though giving a crystalline product, is unpleasant and the yield is low. It would probably be better to use a modification of the following process which we, in fact, applied to the preparation of 4-*secbutyl* phenol. Racemic 4-*hydroxyphenyl-methyl-ethyl carbinol* was dehydrated by heating its benzoate with MgSO_4 , and the re-

Table 1. *The antibacterial activity of 4-substituted 2-aminophenols. Limiting dilution causing complete inhibition of growth (conditions described in text)*

(Figures indicate ml. containing 1 g. of aminophenol causing inhibition for 1 and 7 days.)

Compound in series	Substituent in 4	<i>E. typhosa</i>		<i>Ps. aeruginosa</i>		<i>Staph. aureus</i>	
		1 day	7 days	1 day	7 days	1 day	7 days
(1)	—H	16,000–32,000	16,000	4,000–8,000	2,000	64,000	32,000
(2)	—CH ₃	16,000	8,000	4,000	2,000	32,000	16,000
(3)	—CH ₂ CH ₃	16,000	8,000	4,000	4,000	32,000	16,000
(4)	—(CH ₂) ₂ CH ₃	8,000	8,000	<4,000	<1,000	16,000	8,000
(5)	—CH(CH ₃) ₂	4,000	4,000	—	—	16,000	16,000
(6)	—(CH ₂) ₃ CH ₃	8,000–16,000	8,000–16,000	2,000	<1,000	32,000	16,000
(7)	—CH ₂ CH(CH ₃) ₂	<1,000	—	<1,000	—	64,000	16,000
(8)	—CH(CH ₃)CH ₂ CH ₃	8,000	8,000	<1,000	—	64,000	32,000
(9)	—C(CH ₃) ₃	4,000	4,000	<1,000	—	16,000	8,000
(10)	—CO·CH ₃	4,000	4,000	—	—	32,000	16,000
(11)	—CO·CH ₂ ·CH ₃	2,000	2,000	—	—	64,000	2,000
(12)	—CO(CH ₂) ₂ CH ₃	4,000	2,000	—	—	4,000	4,000

References

Compound 1:	Barber & Haslewood (1944).	Compound 10:	Banks & Hamilton (1939).
" 2:	Heilbron & Bunbury (1943).	" 11:	Edkins & Linnell (1936) (hydrochloride).
" 3, 4, 6:	Baranger (1931)	" 7, 8, 12:	Present work.
" 5, 9:	Stevens & Beutel (1941).		

sulting benzoate of (probably) 4-hydroxy- α -ethylstyrene was hydrogenated. Hydrolysis of the product gave 4-secbutyl phenol in over-all yield (from 4-hydroxy-acetophenone) of about 47%.

In addition to the above carbinol and styrene derivatives, 3-amino-4-hydroxypropiophenone and 3-amino-4-hydroxybutyrophenone have not hitherto been described; 2-amino-4-secbutylphenol and 2-amino-4-isobutylphenol are also new.

EXPERIMENTAL

(A) Assay of antibacterial activity

For testing, weighed amounts of aminophenols were dissolved in water, approx. 0.5N-NaOH, or approx. 0.5N- H_2SO_4 . These solutions were then diluted so as to be 0.02 or 0.01N with respect to NaOH or H_2SO_4 and of concentration 0.2 g./100 ml. with respect to the aminophenol. The following solutions of compounds in Table 1 were used: substances (2) and (10) in water; substances (4), (6), (7), (8) and (9) in 0.01N- H_2SO_4 ; substances (5), (11) and (12) in 0.02N-NaOH. Compounds (1) and (3) were tested in water, in 0.01N- H_2SO_4 , and in 0.02N-NaOH: no difference in the (considerable) activity of either of these substances could be detected when these different solvents were used.

Serial dilutions of each compound were made in broth consisting of: 'Lab-Lemco' (1 g./100 ml.), peptone (British Drug Houses Ltd., 1 g./100 ml.), NaCl (0.5 g./100 ml.) and *p*-aminobenzoic acid (0.005 g./100 ml.) at pH 7.5. An inoculum of 200–500 bacteria from a diluted 18 hr. culture of the organism to be tested was then added to each tube. Certain variations in the medium did not appreciably alter the results, but a great increase in the number of organisms in the inoculum made a considerable difference (see Barber & Haslewood, 1944). The organisms used were: *Eberthella typhosa*, *Pseudomonas aeruginosa* (*Ps. pyocyanea*), and *Staphylococcus aureus*.

(B) Preparation of materials

Elementary micro-analyses were by Mr A. T. Macdonald, Edinburgh. All melting-points are uncorrected. *Abbreviation*: l.p. = light petroleum (b.p. 40–60°).

4-Hydroxy-acetophenone, -propiophenone, -butyrophenone, and -isobutyrophenone were prepared by rearrangement of the appropriate phenol esters under the conditions used by Edkins & Linnell (1936).

4-isoButyl-phenol. 4-Hydroxy-isobutyrophenone (5 ml.) was added to the amalgam made by leaving Zn wool (15 g.) for 5 min. with saturated HgCl_2 solution (excess) and 10N-HCl (1 ml.) and then washing it with water. 5N-HCl (25 ml.) was added at once and the mixture boiled under reflux with more 5N-HCl (12.5 ml. every 30 min.) for 3 hr. Steam was blown through the product and the oil which came over was extracted with ether. After evaporation of the washed and dried ether, the residue was distilled *in vacuo*. The distillate had b.p. 125–126°/11–12 mm. and formed crystals of m.p. 51–52°. (Found: C, 80.1; H, 9.4%. Calc. for $\text{C}_{10}\text{H}_{14}\text{O}$: C, 80.0; H, 9.3%.)

2-Amino-4-isobutylphenol (I: $R = -\text{CH}_2\cdot\text{CH}(\text{CH}_3)_2$). A solution of 4-isobutylphenol (2 g.) in acetic acid (20 ml.) was treated gradually with 15 ml. of a nitrating solution (8.5 g.

NaNO_3 , 2.3 ml. H_2SO_4 , 20 ml. water and acetic acid to make the volume 50 ml.). When a few drops of this had been added the mixture was heated on the water-bath until a change of colour showed that reaction had begun; it was then cooled to c. 20° and nitration continued at that temperature. After 20 hr. steam was blown through the diluted mixture and the distillate was treated with Na_2CO_3 in excess. The filtered solution was acidified with HCl and steam again blown through it. The yellow oil which came over was dissolved, in a volume of c. 300 ml., by the addition of 3 g. Na_2CO_3 . To the boiling solution enough solid $\text{Na}_2\text{S}_2\text{O}_4$ (sodium hyposulphite) was added to discharge the orange colour. The hot liquid was filtered at once, and on cooling at 0°, deposited the aminophenol as leaflets which were collected, washed with cold water and dried at once in the dark *in vacuo* over H_2SO_4 . Yield, 0.7 g. Recrystallization from l.p.-benzene gave bluish leaflets of m.p. 158–159° (decomp.). (Found: C, 72.7; H, 8.7; N, 8.4%. $\text{C}_{10}\text{H}_{13}\text{ON}$ requires C, 72.7; H, 9.1; N, 8.5%.)

2-Amino-4-secbutylphenol (I: $R = -\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$) was prepared from 4-secbutylphenol as above. The product, from l.p.-benzene, had m.p. 127–128° (decomp.). (Found: C, 72.8; H, 8.5; N, 8.5%. $\text{C}_{10}\text{H}_{13}\text{ON}$ requires C, 72.7; H, 9.1; N, 8.5%.)

3-Amino-4-hydroxy-acetophenone (I: $R = -\text{CO}\cdot\text{CH}_3$). 3-Nitro-4-hydroxy-acetophenone (0.4 g.), prepared as described by Edkins & Linnell (1936), was dissolved in water (10–15 ml.) with Na_2CO_3 (0.8 g.). To the boiling solution $\text{Na}_2\text{S}_2\text{O}_4$ (c. 2 g.) was added until the orange colour was discharged. The hot mixture was filtered at once and, at 0°, deposited needles, which were collected, washed with ice-cold water and dried at once in the dark *in vacuo* over H_2SO_4 . Yield, 0.15 g. Recrystallization from water containing a little $\text{Na}_2\text{S}_2\text{O}_4$ and from l.p.-acetone gave almost white needles whose melting-point after drying was 124–125° (decomp.): these still contained water, and were dried *in vacuo* to constant weight before analysis. (Found: C, 63.9; H, 6.1; N, 9.2%. Calc. for $\text{C}_8\text{H}_9\text{O}_2\text{N}$: C, 63.6; H, 6.0; N, 9.3%.) Banks & Hamilton (1939) give the melting-point of this substance as 98°: our preparation had m.p. c. 100° before drying.

3-Amino-4-hydroxy-propiophenone (I: $R = -\text{CO}\cdot\text{CH}_2\text{CH}_3$) was prepared as above. The recrystallized product had m.p. 144–145° (decomp.). (Found: N, 8.4%. $\text{C}_9\text{H}_{11}\text{O}_2\text{N}$ requires N, 8.5%.)

3-Amino-4-hydroxy-butyrophenone (I: $R = \text{CO}\cdot(\text{CH}_2)_2\text{CH}_3$). The corresponding nitro-compound was described by Morgan & Hickinbottom (1921): we made it by the method of Edkins & Linnell (1936). Reduction, as above, gave the aminophenol which, from l.p.-benzene, formed white leaflets, m.p. 115–116° (decomp.). (Found: C, 66.9; H, 7.6; N, 8.05%. $\text{C}_{10}\text{H}_{13}\text{O}_2\text{N}$ requires C, 67.1; H, 7.3; N, 7.8%.)

4-Hydroxyphenyl-methyl-ethyl carbinol. A Grignard reagent, made from ethyl bromide (16.8 g.), ether (100 ml.), and Mg (4 g.), was cooled with water, while a solution of 4-hydroxy-acetophenone (6.8 g.) in dry ether (c. 150 ml.) was added gradually down a reflux condenser. The mixture was thoroughly shaken, with cooling, then boiled for 30 min. under reflux and left at c. 20° for 16 hr. After careful decomposition with water, NH_4Cl (10 g.) and 5N- H_2SO_4 , the product was separated with the ethereal layer, which was washed with water, dried (Na_2SO_4) and evaporated. The residue crystallized with l.p. giving colourless prisms of

m.p. 118–121°. Yield, 6.6 g. A sample, after sublimation at 105°/0.2 mm., had m.p. 120–121°. (Found: C, 72.1; H, 8.8%. $C_{10}H_{14}O_2$ requires C, 72.3; H, 8.4%.)

The above substance (12 g.), dissolved in 5*N*-NaOH (120 ml.), was shaken with benzoyl chloride (25 ml. at once, and 10 ml. after 20 hr.). After 24 hr. the precipitated solid was collected, well washed with water, and dissolved in ether. The ether was dried (Na_2SO_4), filtered and evaporated. The residue, with l.p. formed crystals (17 g.) of m.p. 77–81°. A sample, after sublimation at 145°/0.05–0.1 mm., separated from l.p.-benzene as large colourless prisms, m.p. 83–84°, of 4-benzoyloxyphenyl-methyl-ethyl carbinol. (Found: C, 75.7; H, 6.3%. $C_{17}H_{18}O_3$ requires C, 75.5; H, 6.7%.)

(Probably) 4-Benzoyloxy- α -ethyl-styrene. An intimate mixture of the above benzoate (3 g.) with freshly dried finely powdered anhydrous $MgSO_4$ (12 g.) was heated at 160–170° for 1 hr. Dilution of the cooled product gave a water-insoluble solid, which was collected, washed, and crystallized from methanol, giving almost colourless needles (1.75 g.);

m.p. 111–112°. (Found: C, 80.7; H, 6.7%. $C_{17}H_{18}O_2$ requires C, 80.9; H, 6.4%.)

4-sec-Butyl-phenol. The above compound (2 g.) in benzene (50 ml.) was shaken with H_2 and Pd-black (0.5 g.) until no more H_2 was absorbed. The product, obtained by evaporation of the filtered solution, was an oil at 20°. It was boiled under reflux with *N*-NaOH (40 ml. in approx. 30% v/v ethanol-water) for 3 hr. Ethanol was then removed in steam, and the alkaline residue was acidified with HCl. The oily phenol was separated by steam distillation and formed an aqueous suspension, which crystallized at 0°. Yield, 1.3 g., m.p. 51–53°; Reilly & Hickinbottom (1920) record m.p. 59°.

SUMMARY

1. A series of substituted 2-aminophenols has been tested *in vitro* for antibacterial activity.

2. It is concluded that such activity is not increased but may be decreased by simple nuclear substitution of 2-aminophenol.

REFERENCES

- Banks, C. K. & Hamilton, C. S. (1939). *J. Amer. chem. Soc.* **61**, 357.
Baranger, P. M. (1931). *Bull. Soc. chim. Fr.* **49**, 1213.
Barber, M. & Haslewood, G. A. D. (1944). *Brit. med. J.* **2**, 754.
Bert, L. (1923). *C.R. Acad. Sci., Paris*, **177**, 452.
Edkins, R. P. & Linnell, W. H. (1936). *Quart. J. Pharm.* **9**, 75, 203.
Heilbron, I. M. & Bunbury, H. M. (1943). *Dictionary of Organic Compounds*. London: Eyre and Spottiswoode.
Morgan, G. T. & Hickinbottom, W. J. (1921). *J. chem. Soc.* **119**, 1879.
Niederl, J. B., Niederl, V., Shapiro, S. & McGreal, M. E. (1937). *J. Amer. chem. Soc.* **59**, 1113.
Reilly, J. & Hickinbottom, W. J. (1920). *J. chem. Soc.* **117**, 103.
Stevens, J. R. & Beutel, R. H. (1941). *J. Amer. chem. Soc.* **63**, 308.

A Metabolite of 2-Acetamidofluorene*

By F. BIELSCHOWSKY, *Department of Pathology and Cancer Research Laboratories, University of Sheffield*

(Received 15 May 1945)

Investigation of the fate of carcinogenic and related non-carcinogenic hydrocarbons in animals was begun by Boyland & Levi (1935) and by Chalmers & Peacock (1936), a few years after the discovery of the first carcinogenic compound of known structure (Kennaway, 1930). The presence of phenolic derivatives of carcinogenic hydrocarbons in the excreta of rabbits, rats and mice was recognized at an early stage of these studies, and some of the metabolites were later isolated and identified. Fieser (1941), reviewing the results of this line of cancer research, came to the conclusion that: 'biological hydroxylation represents an avenue for the elimination of carcinogens in a detoxified form', and that 'the reaction of detoxication, if indeed it may be so regarded, involves an attack of the molecule at some site other than the centre of special susceptibility to substitutions'. Relevant data obtained since

1941 conform in the main with Fieser's views. Berenblum & Schoental (1943) called attention to the fact that 'with benzantracene and dibenzanthracene, the positions in the molecule metabolically attacked are also those where sulphonation occurs *in vitro*, provided the most reactive positions are blocked'.

It seemed of interest to find whether these rules applied to 2-acetamidofluorene,* one of the most potent of the carcinogens which produce distant tumours (Wilson, DeEd & Cox, 1941; Bielschowsky, 1944; Armstrong & Bonser, 1944). Wilson *et al.* (1941) observed that the fur of the experimental rats, and the white pine shavings on which they

* Previous authors have described this compound as 2-acetylaminofluorene. It is here described as 2-acetamidofluorene, in conformity with the rules of organic nomenclature adopted.