226. *Some Sulphanilamidophenyl Cyanides and Related Compounds.*

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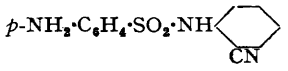
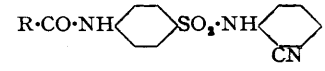
o-, m-, and p-*Sulphanilamidophenyl cyanides*, some derivatives of the *o*-isomeride and three *sulphanilamidomethoxyphenyl cyanides* were prepared and examined for antibacterial activity. Those having an *o*-cyano grouping were superior to other cyanides and to the sulphonamides described in an earlier paper. Their superiority may be correlated with their acid dissociation constants which were also determined.

An earlier paper (Cook, Heilbron, and Reed, this vol., p. 182) described the preparation of some sulphonamides derived from a number of aminophenyl heterocyclic compounds. It was later found that *o*-sulphanilamidophenyl cyanide (I) obtained from the N^4-acetyl derivative (*loc. cit.*) surpasses the compounds described therein as an antibacterial agent. m- and p-*Sulphanilamidophenyl cyanides* have also been prepared but were sub-

stantially inferior to the *o*-isomeride as bacterial inhibitors. This suggested that the *o*-cyano grouping may have a specific effect and a number of further sulphonamides containing this grouping and other substituents in various positions were synthesised.

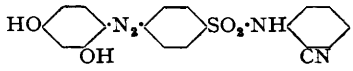


(I.)



(II.)

a, R = ·CH_2·CH_2·CO_2H
b, R = ·CH:CH·CO_2H

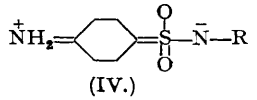


(III.)

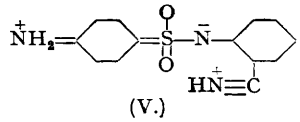
Thus the *o*-sulphanilamidophenyl cyanide was readily converted into the *derivatives* of succinanilic and maleanilic acid (II*a* and II*b*) (cf. Moore and Miller, *J. Amer. Chem. Soc.*, 1942, **64**, 1572) by reaction with the appropriate acid anhydrides; coupling the diazotised sulphonamide with resorcinol gave the azo dyestuff (III) and introduction of the sulphanilyl residue into anthranilamide gave o-*sulphanilamidobenzamide via* its N^4-*acetyl* compound. Reduction of 4-nitro-2-cyanoanisole to 4-*amino*-2-*cyanoanisole* followed by the usual reaction with acetylsulphanilyl chloride gave 5-*sulphanilamido*-2-*methoxyphenyl cyanide via* its N^4-*acetyl* derivative. Similar reactions with 3-nitro-4-aminoanisole and 5-nitro-2-aminoanisole gave in the one case 3-*nitro*- and 3-*amino*-4-*cyanoanisole* and eventually 2-*sulphanilamido*-4-*methoxyphenyl cyanide* and its N^4-*acetyl* compound; in the other case the products were 5-*nitro*- and 5-*amino*-2-*cyanoanisole* and eventually 4-*sulphanilamido*-2-*methoxyphenyl cyanide* and its N^4-*acetyl* derivative. p-*Sulphanilamidobenzyl cyanide* and its N^4-*acetyl* compound were also obtained by orthodox reactions.

The *in vitro* antibacterial activities of many of these sulphonamides and their N^4-acetyl derivatives were kindly examined by the scientific staff of the Department of Experimental Medicine, Glaxo Laboratories, Ltd. The outstanding feature of the results was the demonstration of the superiority of 2-sulphanilamido-4-methoxyphenyl cyanide containing the *o*-cyano grouping over its isomerides and related compounds, though some of the others were not without promise, and a similar remark may be made of the less complete *in vivo* tests. Thus, 2-sulphanilamido-4-methoxyphenyl cyanide inhibited the growth of *B. subtilis* on a synthetic medium at a dilution of 1 : 640,000, and *Hæmolytic Streptococcus* at a dilution of 1 : 320,000 (corresponding figures for sulphathiazole were 1 : 320,000 and 1 : 160,000 respectively). The succinyl derivative of (I) and the azo dyestuff derived from (I) and resorcinol also had high *in vitro* activity against *B. subtilis*, *E. coli*, *Staph. aureus* and *Hæmolytic Streptococcus*; in all cases the antibacterial action as manifested by pellicle inhibition of *B. subtilis* was removed by *p*-aminobenzoic acid.

A connection between the acid dissociation constant of a sulphonamide and its *in vitro* biological activity (Fox and Rose, *Proc. Soc. Exp. Biol. Med.*, 1942, **50**, 142; Schmelkes *et al.*, *ibid.*, 1942, **50**, 145) has led to a number of views on the variation of activity and its correlation with structure. Roblin and Bell (*J. Amer. Chem. Soc.*, 1942, **64**, 2905) pictured the effectiveness of sulphonamides as dependent on the degree to which they are ionised as acids at pH = 7; comparison of a large number of sulphonamides indicated that the most effective were those of $pK_a = 6{\cdot}7$ and it was concluded that, excluding other important considerations governing *in vivo* activity, the maximum activity of N^1-substituted sulphonamides had been reached. Kumler and Daniels (*J. Amer. Chem. Soc.*, 1943, **65**, 2190) have accommodated some apparent discrepancies in the simpler view by emphasising the probable importance of the contribution of a resonating quinonoid dipolar form (IV).



(IV.)



(V.)

In most cases of acidic sulphonamides, however, their acid dissociation constants, or more precisely the degree to which they are ionised at *ca.* pH = 7, afford a measure of the major factor; the negative ion, contributing to the quinonoid form and in this restricted manner might be expected to indicate the effectiveness of the sulphonamides as antibacterial agents. It was of interest therefore to determine the acid dissociation constants of the more promising of the sulphonamides described in this and the former communication. Of these twenty-two were titrated electrometrically in 50% ethanol and from the apparent pK_a values, pK_a values in water were deduced from the curve constructed by Roblin and Bell (*loc. cit.*) relating these constants. The results are summarised in the following table.

It will be seen that this table, arranging the compounds in the order of their decreasing acidity, is headed by the two *o*-sulphanilamidophenyl cyanides, the antibacterial activity of which had been noted as outstanding. It should also be stated that *p*-sulphanilamidobenzyl cyanide, and to a lesser extent some of the other sulphonamides, had more promising antibacterial activities than the compounds arranged in this order might indicate. Disregarding these minor deviations, however, it seems that the activities of this restricted group of sulphonamides may generally be satisfactorily correlated with their acid dissociation constants. In view of the marked superiority of the *o*-sulphanilamidophenyl cyanides over their *m*- and particularly their *p*-isomerides, a specific effect such as a contribution to the quinonoid form by virtue of an influence which in an extreme form may

Dissociation Constants of Sulphonamides.

Sulphonamide.	$pK_{a(50\%\ EtOH)}$.	$pK_{a(H_2O)}$.	K_a.
2-S-4-methoxyphenyl cyanide †	7·97	6·75	$1·8 \times 10^{-7}$
2-S-phenyl cyanide	8·28	7·1	$7·9 \times 10^{-8}$
4-S-methoxyphenyl cyanide	8·4	7·25	$5·6 \times 10^{-8}$
4-S-phenyl cyanide	8·71	7·5	$3·2 \times 10^{-8}$
2-S-benzamide	8·85	7·7	$2·0 \times 10^{-8}$
3-S-phenyl cyanide	9·15	8·0	$1·0 \times 10^{-8}$
2-(*p*-S-phenyl)-4-methylthiazole *	9·56	8·5	$3·2 \times 10^{-9}$
Sulphapyridine	9·81	8·7	$2·0 \times 10^{-9}$
Ethyl 4-(*p*-S-phenyl)-2 : 6-dimethylpyridine-3 : 5-dicarboxylate *	10·2	9·15	$7·1 \times 10^{-10}$
2-(*m*-S-phenyl)-4-methylthiazole *	10·27	9·2	$6·3 \times 10^{-10}$
4-S-benzyl cyanide	10·47	9·5	$3·2 \times 10^{-10}$
α-4-S-benzylpyridine *	10·6	9·6	$2·5 \times 10^{-10}$
Ethyl 4-(*m*-S-phenyl)-2 : 6-dimethylpyridine-3 : 5-dicarboxylate *	10·7	9·7	$2·0 \times 10^{-10}$
α-3-S-phenylpyridine *	10·85	9·9	$1·3 \times 10^{-10}$
Sulphanilamide	11·5	10·65	$2·2 \times 10^{-11}$
5-S-2-methoxyphenyl cyanide	Weakly acidic.		
α-2-S-phenylpyridine *	Weakly acidic.		
α-4-S-phenylpyridine *	Weakly acidic.		
γ-4-S-phenylpyridine *	Weakly acidic.		
2-(*m*-S-phenyl)-4 : 6-dimethylpyrimidine *	Weakly acidic and insoluble.		
4′-S-4-α-pyridyldiphenyl *	Weakly acidic and insoluble.		
2-(*o*-S-phenyl)-4-methylthiazole *	Weakly acidic and insoluble.		

* Cook, Heilbron, and Reed (*loc. cit.*). † S = *p*-$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH$.

possibly be represented by (V) cannot be excluded. Work is in progress to examine the effect of the cyano grouping on the activity of other groups of antibiotic agents.

Experimental.

o-Aminophenyl cyanide was prepared by the method of Bogert and Hand (*J. Amer. Chem. Soc.*, 1902, **24**, 1038); when *o*-nitrophenyl cyanide (13 g.) in ethanol (200 c.c.) was reduced catalytically over Adams' catalyst at room temperature and pressure, reduction was incomplete and the sole product isolated formed needles, m. p. 197—198° from ethanol. It contained no amino group and was evidently *o*-azoxyphenyl cyanide (lit., m. p. 194—195°) (Found: C, 68·0; H, 3·0; N, 22·6; *M*, cryoscopic in camphor, 250. Calc. for $C_{14}H_8ON_4$: C, 67·75; H, 3·25; N, 22·6%; *M*, 248).

o-N^4-Acetylsulphanilamidophenyl cyanide (Cook, Heilbron, and Reed, *loc. cit.*) (10 g.) was refluxed with 2N-hydrochloric acid (100 c.c.) for 1 hour and the solution neutralised whereupon o-*sulphanilamidophenyl cyanide* (8·7 g.) was precipitated; it separated from ethanol or acetone–light petroleum in solvated form but crystallised normally from benzene–ligroin in slender needles, m. p. 157—158° (Found: C, 57·1; H, 4·1. $C_{13}H_{11}O_2N_3S$ requires C, 57·1; H, 4·1%). The sulphonamide (6·8 g.) was boiled with 2N-hydrochloric acid (31 c.c.) and water (100 c.c.) and the cold solution diazotised over 30 minutes with sodium nitrite (1·8 g.) in water (12 c.c.). After stirring for 3 hours, the yellow suspension was coupled by adding it to resorcinol (2·75 g.) in excess of 2N sodium hydroxide. The *azo dyestuff* was precipitated with 2N hydrochloric acid and was purified by solution in alkali and reprecipitation, or by solution in ethanol and precipitation with water. It formed a dark red powder melting rather indefinitely at 170° (Found: N, 14·5. $C_{19}H_{14}O_4N_4S$ requires N, 14·2%). Equimolecular quantities of maleic anhydride and 2-sulphaniloamidophenyl cyanide were boiled in acetone for 2—3 minutes and solvent removed. Solution of the residue in aqueous sodium bicarbonate and acidification gave the crude derivative in almost theoretical yield.

The 4-*sulphon*-(2′-*cyanophenyl*)*amide* of maleanilic acid separated from ethanol in microcrystalline granular form, m. p. 189° (Found: C, 55·0; H, 3·55. $C_{17}H_{13}O_5N_3S$ requires C, 55·0; H, 3·5%). 2-Sulphanilamidophenyl cyanide (9·5 g.), succinic anhydride (5 g.) and dioxan (20 c.c.) were refluxed for 30 minutes. Part of the product (2·8 g.) crystallised from the hot filtrate, and a further quantity (5·5 g.; total 65%) was obtained by removing solvent, extracting the residue with aqueous sodium bicarbonate, and acidifying the extract. The 4-*sulphon*-(2′-*cyanophenyl*)*amide* of succinanilic acid separated from aqueous ethanol or ethanol–ligroin in microcrystalline form, m. p. 203° (Found: C, 54·5; H, 4·25. $C_{17}H_{15}O_5N_3S$ requires C, 54·7; H, 4·1%).

Acetylsulphanilyl chloride (22·5 g.) was added to *m*-aminophenyl cyanide (Bogert and Hand, *J. Amer. Chem. Soc.*, 1904, **26**, 468) (11·3 g.) in pyridine (25 c.c.) and the reaction completed on the steam-bath for 1 hour. Water precipitated m-N^4-*acetylsulphanilamidophenyl cyanide* which was used directly in the succeeding stage; it crystallised from acetone–ligroin in prisms, m. p. 232—233° (Found: C, 57·4; H, 4·3. $C_{15}H_{13}O_3N_3S$ requires C, 57·1; H, 4·2%). The crude acetyl derivative was refluxed for 1 hour with 2N-hydrochloric acid and the solution neutralised. The product crystallised from acetone–light petroleum (charcoal) and m-*sulphanilamidophenyl cyanide* (15 g.; 57% overall) separated in glistening plates (Found: C, 57·3; H, 3·9. $C_{13}H_{11}O_2N_3S$ requires C, 57·1; H, 4·1%).

p-Aminophenyl cyanide (Bogert and Hand, *ibid.*, 1910, **32**, 1495) was similarly converted into p-N^4-*acetylsulphanilamidophenyl cyanide* which separated from acetone–ligroin in prismatic needles, m. p. 246—247° (Found: C, 57·1; H, 4·35. $C_{15}H_{13}O_3N_3S$ requires C, 57·1; H, 4·2%), and thence, as above, into p-*sulphanilamidophenyl cyanide*; the latter separated from acetone–ligroin in large needles, m. p. 175—176° (yield, 65% overall) (Found: C, 57·0; H, 4·2. $C_{13}H_{11}O_2N_3S$ requires C, 57·1; H, 4·1%).

Anthranilamide was conveniently prepared by shaking methyl anthranilate (3·0 g.) with ammonia (*d* 0·88, 25 c.c.) until the oil disappeared (several days), removing solvent and crystallising the residue from water; this was less troublesome than the process using liquid ammonia (cf. Morris, Handford, and Adams, *J. Amer. Chem. Soc.*, 1935, **57**, 953). o-N^4-*Acetylsulphanilamidobenzamide* was obtained (yield, 90%) in the usual way; it crystallised from *cyclo*hexanone in small prisms, m. p. 261° (Found: C, 54·05; H, 4·6. $C_{15}H_{15}O_4N_3S$ requires C, 54·05; H, 4·6%). Refluxing for 4½ hours with 10 vols. of 2N-hydrochloric acid and neutralising the solution gave o-*sulphanilamidobenzamide* which crystallised from ethanol–ligroin in needles, m. p. 175° (Found: C, 53·8; H, 4·5. $C_{13}H_{13}O_3N_3S$ requires C, 53·6; H, 4·5%).

p-N^4-*Acetylsulphanilamidobenzyl cyanide* was prepared from *p*-aminobenzyl cyanide in the usual way; it separated from ethanol–ligroin (charcoal) in small needles, m. p. 213° (Found: C, 58·3; H, 4·55. $C_{16}H_{15}O_3N_3S$ requires C, 58·35; H, 4·6%). Refluxing the crude acetyl compound with 10 vols. of 2N-hydrochloric acid for 1 hour and neutralising the

solution gave p-*sulphanilamidobenzyl cyanide* (yield, 73% overall), which separated from acetone–ligroin in needles, m. p. 201° (Found: C, 58·7; H, 4·7. $C_{14}H_{13}O_2N_3S$ requires C, 58·5; H, 4·55%).

4-Nitro-2-cyanoanisole (1·7 g.) in ethyl acetate (200 c.c.) was hydrogenated at room temperature and pressure over Adams' catalyst, the theoretical quantity of hydrogen for reduction of the nitro to the amino group being fairly rapidly absorbed. Removal of solvent and crystallisation of the residue from ethyl acetate–ligroin gave 4-*amino*-2-*cyanoanisole* in pale yellow needles, m. p. 100° (Found: C, 64·6; H, 5·6. $C_8H_8ON_2$ requires C, 64·8; H, 5·4%). Treatment of the amino-cyanide (11·2 g.) with acetylsulphanilyl chloride (17·7 g.) in pyridine (50 c.c.) as in previous preparations gave 5-N^4-*acetylsulphanilamido*-2-*methoxyphenyl cyanide* (2·3 g.; 85%), which crystallised from ethanol in small needles, m. p. 237—238° (Found: C, 55·6; H, 4·1. $C_{16}H_{15}O_4N_3S$ requires C, 55·6; H, 4·1%). Boiling the acetyl derivative with 15 vols. of 2N-hydrochloric acid for 3 hours and neutralising the solution gave the crude sulphonamide (8·2 g. or 45% of theory). 5-*Sulphanilamido*-2-*methoxyphenyl cyanide* separated from acetone–ligroin in small prisms, m. p. 219° (Found: C, 55·45; H, 4·35. $C_{14}H_{13}O_3N_3S$ requires C, 55·45; H, 4·3%).

3-Nitro-4-aminoanisole (68 g.) was dissolved with warming in concentrated hydrochloric acid (94 c.c.) and water (150 c.c.). The cold suspension of hydrochloride was diazotised at 0° with sodium nitrite (28 g.) in water (100 c.c.) over 45 minutes and after a further 30 minutes the diazonium salt solution was added over 30 minutes to a stirred boiling solution prepared from potassium cyanide (112 g.) and copper sulphate (102 g.) in water (500 c.c.). Filtration of the warm liquid gave the crude cyanide (42 g.), m. p. 137—138°. 3-*Nitro*-4-*cyanoanisole* separated from ethyl acetate–ligroin in yellow-brown plates, m. p. 140° (Found: C, 54·1; H, 3·5. $C_8H_6O_3N_2$ requires C, 53·95; H, 3·4%) (this compound is mentioned but not described in F.P. 828,202). The preceding compound (40 g.) was added during 20 minutes to stannous chloride (168 g.) in concentrated hydrochloric acid (500 c.c.), the temperature being not higher than 40°. After the reaction was completed, the solution was heated to 65° and a small red precipitate separated and rejected; the filtrate was set aside at −12° for several hours and the crystalline deposit separated and added to water (100 c.c.) and 2N-sodium hydroxide (300 c.c.). The free base was extracted with ether and solvent removed (21·3 g., 65%). 3-*Amino*-4-*cyanoanisole* crystallised from ethyl acetate–ligroin in colourless plates, m. p. 96° (Found: C, 64·6; H, 5·45. $C_8H_8ON_2$ requires C, 64·85; H, 5·45%) (this compound is mentioned without details of preparation or description in B.P. 483,585). The amine (19·7 g.) was treated with acetylsulphanilyl chloride (31 g.) in pyridine, as in previous preparations. Crude 2-N^4-*Acetylsulphanilamido*-4-*methoxyphenyl cyanide* (38·5 g., 84%) separated from ethanol in short needles, m. p. 185° (Found: C, 55·8; H, 4·4. $C_{16}H_{15}O_4N_3S$ requires C, 55·6; H, 4·35%). The acetyl compound required 4 hours' refluxing with 10 vols. of 2N-hydrochloric acid to effect solution and the sulphonamide was precipitated on neutralisation. 2-*Sulphanilamido*-4-*methoxyphenyl cyanide* separated from ethanol–ligroin in needles, m. p. 182° (Found: C, 55·6; H, 4·6. $C_{14}H_{13}O_3N_3S$ requires C, 55·45; H, 4·3%).

5-Nitro-2-aminoanisole (67·2 g.) in 2N-hydrochloric acid (500 c.c.) was diazotised over 20 minutes with sodium nitrite (28 g.) in water (100 c.c.) and the solution added over 1 hour to boiling cuprocyanide solution (112 g. of potassium cyanide, 102 g. of copper sulphate, and 500 c.c. of water); extraction of the filtered sludge with ethanol and allowing the extract to crystallise gave 5-*nitro*-2-*cyanoanisole* (15 g.). It separated from ethanol, acetone–ligroin, or ethyl acetate–ligroin in golden orange needles, m. p. 180° (Found: C, 54·1; H, 3·6. $C_8H_6O_3N_2$ requires C, 53·9; H, 3·4%); a further quantity was obtained by extracting the sludge with acetone (total yield, 45 g., 63%). The nitro compound, reduced with stannous chloride as in the case of the preceding isomeride, was converted into the crude amino compound (80%); 5-*amino*-4-*cyanoanisole* crystallised from water in needles, m. p. 102° (Found: C, 64·75; H, 5·5. $C_8H_8ON_2$ requires C, 64·85; H, 5·45%). The sulphanilyl residue was introduced exactly as in the preceding preparation. 4-N^4-*Acetylsulphanilamido*-2-*methoxyphenyl cyanide* (80%) separated in small crystals, m. p. 242°, from acetone–ligroin (charcoal) (Found: C, 55·6; H, 4·4. $C_{16}H_{15}O_4N_3S$ requires C, 55·6; H, 4·35%). 4-*Sulphanilamido*-2-*methoxyphenyl cyanide* (80%) separated from ethanol in needles, m. p. 210° (Found: C, 55·7; H, 4·3. $C_{14}H_{13}O_3N_3S$ requires C, 55·45; H, 4·3%).

The sulphonamides (1—4 mg.) in CO_2-free 50% ethanol (1 c.c.) were titrated electrometrically with 0·05N-sodium hydroxide in 50% ethanol using the apparatus described by Catch, Cook, and Kitchener (this vol., p. 319). The equivalent values were in agreement with those calculated, and the apparent pK_a values in 50% ethanol were reduced to pK_a values in water alone (see Roblin and Bell, *loc. cit.*). The reliability of this procedure was indicated by its application to two known sulphonamides. Found (for sulphapyridines): pK_a (H_2O) = 8·7; Schmelkes *et al.* (*loc. cit.*) quote 8·29, Roblin and Bell (*loc. cit.*) 8·4, Fox and Rose (*loc. cit.*) 8·5, Wollfbrandt (*Dansk Tidsskr. Farm.*, 1940, **14**, 113; *J. Amer. Pharm. Assoc., Pharm. Abs.*, 1942, **21**, 29) 8·7. Found (for sulphanilamide): 10·65; Albert and Goldacre (*Nature*, 1942, **149**, 245) quote 10·2, Roblin and Bell 10·43, Fox and Rose 10·5, Schmelkes *et al.* 10·66.

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