SULPHANILAMIDES

Part I. Salts with Sulphonic Acids

BY S. K. MUNSHI, B. D. TILAK AND K. VENKATARAMAN, F.A.SC.

(Department of Chemical Technology, University of Bombay)

Received January 26, 1948

CERTAIN aspects of the work carried out in this laboratory on the structural characteristics of organic compounds which lead to surface activity in relation to their use in textile processing[1] suggested an extension of these investigations in the field of chemotherapy. The adsorption of wetting agents and detergents by textile fibres indicates that a combination of surface-active compounds with drugs would probably lead to more active chemotherapeuticals, since affinity of a substance for bacteria has been considered as a requisite for its chemotherapeutic activity. The bactericidal and lytic action of a number of surface-active agents,[2] the potentiation of drugs by synergistic action with detergents,[3] and the activity of drugs modified in their chemical constitution by the introduction of structural features which lead to surface activity[4] have been studied in recent years.

The arylamine salts of aromatic sulphonic acids are useful in the characterisation of both the amines and the sulphonic acids,[5] and the object of the present work is to synthesise surface-active compounds from wetting agents containing sodium sulphonate groups and different sulphanilamides. Arylamine salts of sulphonic acids are relatively stable to mineral acids, but they break down on treatment with alkali and can in fact be estimated by titration with caustic soda solution. By virtue of their stability to mineral acids, the sulphanilamide salts should pass through the stomach, and under the alkaline condition in the intenstines liberate the drug component. Such an action would probably make these salts active intestinal bacteriostatic agents. The surface-active component should also lead to advantages due to the spreading, penetration, and emulsification properties associated with it.

Sulphanilamide salts of several aliphatic carboxylic acids, and aromatic, sulphonic and carboxylic acids have been mentioned[6–10] but do not appear to have been isolated in pure form and characterised. The bacteriostatic activity of some of the salts has been stated to be better than sulphanilamide, although the salts contained only 40 to 50 per cent. of the sulpha component.

Before attempting the synthesis of salts from surface-active agents, a preliminary study of salts prepared from naphthalene-2-sulphonic acid and

a series of sulphanilamides was undertaken. The salts were prepared by adding a boiling aqueous solution of the sodium salt of the sulphonic acid (one mole) to a hot solution of the sulphanilamide (one mole) in water containing hydrochloric acid (1·4 moles), and boiling the contents for about two minutes. The salts generally separated quickly on cooling, although in some cases it was necessary to leave the mixture overnight or even longer or to concentrate it to smaller bulk.

The salts of sulphanilamide and sulphathiazole were easily prepared, but salt formation in the case of sulphanilamides with basic N^1-substituents such as sulphapyridine, sulphadiazine and sulphaguanidine proved to be difficult in the early experiments in which the equivalent quantity of hydrochloric acid was used. In order to find out whether the basic N^1-substituents were in any way responsible for the failure of the N^4-amino groups to form ammonium salts, a series of salts of naphthalene-2-sulphonic acid with 2-aminothiazole, 2-aminopyrimidine, and 2-amino-4:6-dimethylpyrimidine were prepared. In the preparation of these salts, it was found that addition of excess of hydrochloric acid was necessary for salt formation. Using excess of hydrochloric acid (up to 4 moles) naphthalene sulphonic acid salts of sulphapyridine, sulphadiazine, sulphamerazine, sulphaguanidine, N^1-acetylsulphanilamide, and *p*-aminobenzoic acid were readily prepared. Crystallisation of most of these salts was effected from dilute hydrochloric acid. To get a more soluble product the salt of sulphanilamide with phenol-*p*-sulphonic acid was prepared, and was found to melt at 241–42° (decomp.) as against 216–220° (decomp.) recorded in literature.[7] A mixture of the authentic salt and sulphanilamide hydrochloride gave the m.p. 216–20° (decomp.), showing that the compound described earlier was probably such a mixture.

The purity of the salts was estimated by titration with standard sodium hydroxide, in addition to elementary analysis. Sulphanilamide was not acidic to phenolphthalein, and its salt with naphthalene-2-sulphonic acid required one equivalent of sodium hydroxide corresponding to the ammonium salt linkage. Sulphathiazole was acidic to phenolphthalein and not acidic to methyl red. Its salt with naphthalene-2-sulphonic acid, when titrated against standard alkali using methyl red, therefore, required one equivalent of alkali corresponding exclusively to the salt linkage; using phenolphthalein the salt titrated for two equivalents of alkali corresponding to both the ammonium salt and the sulphonamido groups. The purity of the salts from other sulphanilamides were not determined by titration as they could not be obtained entirely free from traces of hydrochloric acid.

The formation of salts of arylamines with wetting agents containing a sodium sulphonate or a sulphate group has been applied for the estimation of fatty alcohol sulphates and the sulphonates.[11] Judging from the ease with which aromatic sulphonic acids such as phenol-*p*-sulphonic and naphthalene-2-sulphonic acid gave salts with the different sulphanilamides, one of the first wetting agents which was used for salt formation with sulphanilamide was dibutylnaphthalene sodium sulphonate (Nekal BX, I. G. Farbenindustrie). While most of the alkali salt merely decomposed to the free alkylnaphthalenesulphonic acid under the acid conditions of the reaction, a small quantity of the desired ammonium salt could be isolated and characterised. Sodium dioctylsulphosuccinate (Aerosol OT, American Cyanamid Co.) gave a light brown hydroscopic salt with sulphanilamide. It could not be crystallised, but the analysis and the properties of the substance gave definite indication of ammonium salt formation. 4-Laurylamidotoluene-2-sodium sulphonate,[12] which is a powerful wetting agent, gave a hydroscopic amorphous light brown salt with sulphanilamide possessing very good wetting power, and this compound was obtainable in analytically pure condition.

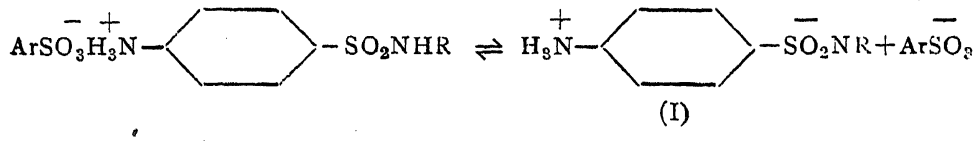
With the object of determining the relative ease with which *p*-aminobenzoic acid (an essential growth factor for the bacteria-enzyme system) and sulphanilamide combine with organic anions such as the sulphonic ion, the salt of sulphanilamide and naphthalene-2-sulphonic acid was treated with *p*-aminobenzoic acid under various conditions. While *p*-aminobenzoic acid replaces sulphanilamide appreciably at elevated temperature (about 100°) to give the salt of the former with the sulphonic acid, replacement could be noticed to a small extent even at room temperature.

Bacteriostatic Activity of the Ammonium Salts

The *in vitro* tests indicated that the salts are more active than the parent sulphanilamide in spite of the lower content (45–56%) of the active sulpha components. Sulphanilamides or naphthalene-2-sodium sulphonate when tested separately in the amounts present in the salts are inactive. The enhanced potency of the salts cannot be due to increased solubility as mentioned by Smyth and Carpenter,[6] because the salts are less soluble as compared to the sulpha components.

Since Woods and Fildes[13] first suggested that *p*-aminobenzoic acid is an essential metabolite necessary for bacterial growth and that the bacteriostatic action of sulphanilamides is due to their ability to block the enzyme system or systems in competition with *p*-aminobenzoic acid, various hypotheses[14–16] based on this generalisation have been advanced as regards the mode of action and the varying potency of different sulphanilamides. The

increased activity of the ammonium salts now prepared suggests that in sulphanilamides and *p*-aminobenzoic acid both the acidic and basic parts of the molecule are involved in the bacteriostatic activity. A sulphanilamide should then have maximum activity if the basic and acidic ends of the molecule are rendered more reactive by ionisation leading to a zwitterionic structure. The potentiation of sulphoanilamides by ammonium salt formation could therefore be due to the fact that by ionisation the sulpha components would acquire the more reactive zwitterion form (I).



(I)

Klotz and Gruen[17] have shown that zwitterion formation in sulphanilamide is negligible, but *p*-aminobenzoic acid is present as zwitterions to the extent of 3 per cent. at pH 7. Bell and Roblin[14] obtained indirect evidence to suggest that the ionised carboxyl group in *p*-aminobenzoic acid increased the basicity of the amino group threefold. Recently, Fuller[18] has suggested that the activity of sulphanilamides is determined both by the acidic sulphonamide group and the basic amino group.

The acidic and the basic ends of the sulphanilamide molecules probably enter into complex formation with the basic and acidic active centres of the bacterial enzyme, either separately or at the same time, since the latter is amphoteric in character and contains both positively and negatively charged groups between the extreme limits of pH 2–13 like many other proteins.[19]

In the light of the relation between metabolites and their structurally related antagonists,[20] the formation of complexes by sulpha drugs (antagonists) in competition with complex-formation by *p*-aminobenzoic acid (essential metabolite) will depend on the relative strength of the electrical charges on *p*-aminobenzoic acid and the sulpha drugs, as also on the charges at the points of attachment in the enzyme.

When the ammonium salt ionises ultimately to (I), the zwitterionic character of the latter will be further stabilised by complementary inductive effects of the positively charged ammonium and negatively charged sulphonamide groups. The increased activity of the ammonium salts could be explained on the basis of increased attraction between the active centres in the enzyme and the zwitterions (I) of the sulphanilamide salts, as compared to the parent sulphanilamides, the increased attraction being due to the increased electrical charges on the amino and the sulphonamido groups in the form (I). Under optimum conditions these attractive forces may be comparable in strength to the attractive forces between the essential meta-

bolite, *p*-aminobenzoic acid, and the active centres in the enzyme system. As mentioned earlier, it has been found that the replacement of sulphanilamide from its salts with naphthalene-2-sulphonic acid takes place to some extent even at room temperature. The sulphonic anion might therefore make a small contribution to the bacteriostasis by removing part of the essential metabolite, *p*-aminobenzoic acid, by salt formation, as also by complex formation with the positively charged parts of the enzyme molecule. Inactivation of sulpha drugs *in vivo* during metabolism by N^4-acetylation might perhaps be prevented or minimized by the use of the sulphanilamide salts instead of the parent sulpha componets.

The variation in the activity of different N^1-substituted sulphanilamides could be explained on the basis of the difference in the contribution of the zwitterionic form due to variation in the acidity of sulphonamido groups depending on the N^1-substituent.[14] The greater the acidity of the sulphonamido group, the greater will be its inductive effect on the positive charge of the N^4-ammonium group. It is in fact conceivable that with high acidity of the sulphonamido group, its ionisation might precede the formation of (I). The effect of changes in pH on the activity of the sulphanilamides and on the antagonising power of *p*-aminobenzoic acid (*cf.*, Fuller[18]) could also be explained similarly.

Compounds derived by N^1-substitution of sulphanilamides include most of the therapeutically important sulpha drugs, susbtitution in other positions generally leading to inactive products. The sulphonamide salts now prepared are more active than the parent sulpha drug in spite of the modification of the N^4-amino group. The physiological properties of the sulphanilamides, such as absorption-excretion characteristics, toxicity and easy accessibility to the site of infection, could also be modified by varying the nature of the sulphonic anion in the salts.

Experimental

General Method

Sulphanilamide salt of naphthalene-2-sulphonic acid.—A hot aqueous solution naphthalene-2-sodium sulphonate (4·6 g. in 100 c.c.) was added to a hot solution of sulphanilamide (3·44 g.) in water (20 c.c.) and concentrated hydrochloric acid (2·5 c.c.). After boiling for about 2 minutes and decolourising (norit), the solution was cooled gradually to room temperature when lustrous white flakes of the salt separated, m.p. 262–63° C. (5·0 g.); raised to 263–64° C. on further recrystallisation from hot water (Found: N, 7·3; S, 17·2. $C_{16}H_{16}O_5N_2S_2$ requires N, 7·4; S, 16·8%. 0·200 g. required 5·3 c.c. of 0·1 N sodium hydroxide. Calc.: 5·2 c.c.).

Sulphathiazole salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (4·6 g.) sulphathiazole (5·1 g.), concentrated hydrochloric acid (2·5 c.c.), and water (250 c.c.) gave the salt (8·0 g.) which, crystallised in white needles from water, m.p. 214–15° C. [Found: N, 9·2; S, 20·5. $C_{19}H_{17}O_5S_3N_3$ requires N, 9·1; S, 20·7%. 0·200 g. required 4·2 c.c. (methyl red) and 8·6 c.c. (Alkali Blue 6B) of 0·1 N sodium hydroxide. Calc., 4·3 c.c. and 8·6 c.c. respectively.]

2-Aminothiazole salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (0·92 g.), 2-aminothiazole (0·4 g.), 1·5 N hydrochloric acid (5·27 c.c.), and water (5·0 c.c.) gave the salt (0·68 g.) which after two crystallisations from water gave colourless hexagonal tablets, m.p. 162–64° (Found: N, 8·9. $C_{13}H_{12}O_3N_2S_2$ requires N. 9·1%).

2-Aminopyrimidine salt of naphthalene-2-sulphonic acid.—The salt was prepared as above from 2-aminopyrimidine (0·37 g.). The reaction mixture after leaving overnight and concentration gave the salt (0·625 g.) which after three recrystallisations from water separated as colourless short needles, m.p. 190–192° C.; mixed with 2-aminopyrimidine hydrochloride (m.p. 196° C.) it gave m.p. 160–170° (Found: N, 13·2; S, 10·1. $C_{14}H_{13}O_3N_3S$ requires N, 13·9; S, 10·5%).

2-Amino-4: 6-dimethylpyrimidine salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (4·6 g.), 2-amino-4: 6-dimethylpyrimidine (2·46 g.), concentrated hydrochloric acid (5·0 c.c.), and water (40 c.c.) after boiling, concentration and cooling gave the salt as white needles, m.p. 107–10° C. (8·5 g.); raised to 155–56° after drying in vacuum at 100°. After two recrystallisations from water the anhydrous salt melted at 159–60° C. (Found: N, 11·7; S, 9·4. $C_{16}H_{17}O_3N_3S$ requires N, 12·6, S, 9·6%).

Sulphapyridine salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (2·3 g.), sulphapyridine (2·48 g.), concentrated hydrochloric acid (2 c.c.), and water (30 c.c.) gave the salt partly as an oil and partly as a crystalline solid on cooling. The oil solidified on cooling in a freezing mixture and the combined solid (2·8 g.) was crystallised from alcohol when the salt separated as lustrous colourless needles; dried at room temperature for 3 hours at 5 m.m., m.p. 70–75° C. (Found: N, 8·34. $C_{21}H_{19}O_5N_3S_2$, C_2H_6O requires N, 8·35%). The salt after drying at 100° C. under reduced pressure gave a cream coloured powder, m.p. 152–53° (Found: N, 9·6. $C_{21}H_{19}O_5N_3S_2$ requires N, 9·2%).

Sulphadiazine salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (2·3 g.), sulphadiazine (2·5 g.), concentrated hydrochloric acid (4·0 c.c.), and water (55 c.c.) after boiling and cooling to 30–

35° C. gave the salt as lemon yellow needles, m.p. 188–89° (3·72 g.). The mother liquor on cooling in ice gave a further quantity of the salt as lustrous cream coloured short needles, m.p. 188° (0·55 g.) (Found: N, 11·9; S, 13·6. $C_{20}H_{18}O_5N_4S_2$ requires N, 12·2; S, 14·0%). The salt readily dissociated in water and recrystallisation could only be effected from acidulated water.

Sulphamerazine salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (4·6 g.), sulphamerazine (5·28 g.) concentrated hydrochloric acid (5 c.c.), and water (50 c.c.) gave the salt as cream coloured needles, m.p. 117–20° (9·5 g.); raised to 118–20° on further recrystallisation from acidulated water (Found: S, 13·0; $C_{21}H_{20}O_4N_4S$ requires S, 13·5%).

Sulphaguanidine salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (2·3 g.), sulphaguanidine (2·14 g.), concentrated hydrochloric acid (1 c.c.), and water (35 c.c.) were boiled together and left overnight and the salt which separated as colourless flat needles (3·4 g.) was recrystallised from acidulated water. After drying at 120–30°/150 m.m. it softens at 186° and melts at 225–26° (Found: N, 13·1; S, 15·8. $C_{17}H_{28}O_5N_4S_2$ requires N, 13·3; S, 15·2%).

N^1-Acetylsulphanilamide salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (2·3 g.), N^1-acetylsulphanilamide (2·14 g.), con-concentrated hydrochloric acid (1 c.c.), and water (30 c.c.) after boiling and cooling gave lustrous flakes of the salt, m.p. 250–55° C. (1·65 g.); raised to 253–54° after recrystallisation from acidulated water (Found: N, 7·0; S, 15·5. $C_{18}H_{18}O_6N_2S_2$ requires N, 6·3; S, 15·1%).

p-Aminobenzoic acid salt of naphthalene-2-sulphonic acid.—Naphthalene-2-sodium sulphonate (1·15 g.), *p*-aminobenzoic acid (0·7 g.), concentrated hydrochloric acid (0·5 c.c.), and water (15 c.c.) gave lustrous flakes of the salt; soften at 252°, m.p. above 260° (decomp.) (Found: N, 3·9. $C_{17}H_{15}O_5$ NS requires N, 4·1%).

Sulphanilamide salt of phenol-p-sulphonic acid.—The sulphonic acid (8·7 g.) was dissolved in dilute aqueous sodium hydroxide (2·1 g. in 50 c.c.) and the solution was boiled with sulphanilamide (8·6 g.), concentrated hydrochloric acid (7·0 c.c.), and water (70 c.c.). White needles of the salt separated on cooling, m.p. 240–41° (11·0 g.); raised to 241–42° on further recrystallisation from acidulated water (Found N, 8·3. $C_{12}H_{14}O_6N_2S_2$ requires N, 8·1%.)

Sulphanilamide salt of Nekal BX.—Nekal BX (9·42 g.), sulphanilamide (5·16 g.), concentrated hydrochloric acid (3·65 c.c.), and water (100 c.c.) were boiled together and the solution was cooled in ice when a heavy oil

separated. The clear supernatent liquid was decanted and concentrated, and after cooling for a week in the refrigerator gave white flakes of the salt, m.p. 195–200° C. (3·0 g.). After recrystallisation from acidulated water it gave m.p. 220–222° (Found: N, 5·9. $C_{24}H_{35}O_3N_2S_2$ requires N, 5·7%). The heavy oil from the crude reaction mixture analysed for dibutylnaphthalene sulphonic acid.

Sulphanilamide salt of Aerosol OT.—Aerosol OT (13·2 g.), sulphanilamide (5·15 g.), 1·5 N hydrochloric acid (60 c.c.), and water (150 c.c.) were boiled together and the mixture evaporated to dryness and the residue was extracted in a soxhlet with absolute alcohol. The alcoholic extract gave a cream coloured viscous solid after removal of the solvent analysis of which indicates the formation of the salt.

Sulphanilamide salt of 4-laurylamidotoluene-2-sodium sulphonate.—The wetting agent (5·9 g.), sulphanilamide (2·5 g.), concentrated hydrochloric acid (1·65 c.c.) and water (120 c.c.) were boiled together and cooled when a thick jelly was obtained. It was evaporated to dryness and the residue was extracted with absolute alcohol in a soxhlet and the alcoholic solution on evaporation gave the salt as a light brown hydroscopic powder (Found: N, 8·1; S, 11·2. $C_{25}H_{39}O_6N_3S_2$ requires N, 7·7; S, 11·8%).

Replacement of sulphanilamide from its salt with naphthalene-2-sulphonic acid by p-aminobenzoic acid: Method A.—The sulphanilamide salt (0·38 g.), *p*-aminobenzoic acid (0·137 g.), and water (20 c.c.) were boiled under reflux for one hour. The solution was cooled in ice when lustrous flakes separated (0·045 g.) which after recrystallisation gave m.p. above 260° (decomp.) undepressed when mixed with the salt of *p*-aminobenzoic acid with naphthalene-2-sulphonic acid.

Method B.—A solution of the sulphanilamide salt (0·38 g.) and *p*-aminobenzoic acid (0·137 g.) in water (60 c.c.) was left at room temperature (26-29° C.) for 10 days. The light brown solid obtained by evaporation at room temperature was stirred with water (16 c.c.) and filtered. The residue (0·105 g.) after recrystallisation was found to be the unreacted sulphanilamide salt. The mother liquor was clarified (norit), concentrated and cooled when pale brown needles, m.p. 175–85° (decomp.) were obtained; raised to m.p. above 260 (decomp.) by recrystallisation from water, and undepressed when mixed with the salt of *p*-aminobenzoic acid.

Method C.—A solution of the sulphanilamide salt (0·38 g.) and *p*-aminobenzoic acid (0·137 g.) in 1 per cent. sodium hydroxide (16 c.c.) was acidified with concentrated hydrochloric acid (0·5 c.c.). Small rods separated, m.p. 260–67° (0·175 g.), which were identical with the unreacted

sulphanilamide salt. The filtrate was cooled in ice when lustrous colourless flakes separated, m.p. above 260° (decomp.), undepressed when mixed with the salt of *p*-aminobenzoic acid. The experiment indicates the competitive reactivity of *p*-aminobenzoic acid and sulphanilamide for organic anions such as naphthalene-2-sulphonic in forming ammonium salts.

Bacteriostatic activity of the salts.—The sulphonamides and their salts were tested for bacteriostatic activity, *in vitro* against *E. coli* and *Staphylococcus aureus*, using the following media: (1) 1 per cent. casein hydrolysate, (2) Muir's synthetic medium[21] containing 1 per cent. casein hydrolysate, and (3) beef extract (0·4%) (Lab-Lemco, Oxo-Ltd., London), peptone (1%) and sodium chloride (0·5%).

The tests were carried out at pH 7·6 at 37° and the growth of the bacteria was followed visually by the appearance of turbidity. The concentration of the drug in milligrams per cent. required to prevent the growth after 72 hours was determined. When Muir's synthetic medium alone was used no growth was observed for *Staphylcoccus aureus*. Satisfactory results were, however, obtained when the medium (2) was used. The results of the tests on the salts of sulphonamides with naphthalene-2-sulphonic acid using the three media are given in the following table:

Sulpha drug	Per cent. sulpha drug in the salt	Medium	Minimum effective concentration mg.%			
			Staph. aureus		*E. coli*	
			Salt	Parent drug	Salt	Parent drug
Sulphanilamide ..	45·3	1	500	500	500	500
		2	400	600	500	500
		3	1050	1050	500	800
Sulphathiazole ..	55·1	1	8	12	8	10
		2	8	10	8	12
		3	300	400	400	500
Sulphapyridine ..	54·4	1	12	22	14	22
		2	14	22	12	22
Sulphadiazine ..	54·3	1	8	20	10	22
		2	12	20	10	22
Sulphamerazine ..	56·1	1	20	40	30	30
		2	20	40	30	30
Sulphaguanidine ..	50·7	1	250–300	250	250	300
		2	250	250–300	250	250–300
N^1-Acetylsulphanilamide ..	50·7	1	150	200	150	225
		2	150	200	175	225
		3	400	500	500	500

The results for the bacteriostatic activity of sulphonamides using inhibitor-free media 1 and 2 were comparable with those obtained by other workers.[14] The salts proved to be more active than their sulpha components although they contained only 45–56 per cent. of the sulpha drug. When medium (3) was employed considerable quantities of the sulpha drugs and their salts were required and the inhibitory confentration for sulphapyridine, sulphadiazine, sulphamerazine and sulphaguanidine and for their respective salts could not be determined due to lack of solubility. In medium (3) the salts showed nearly equal bacteriostatic activity in spite of the divergent activity of the sulpha components. The tests on the salts derived from wetting agents could not be carried out due to insufficient quantity of the analytically pure materials.

We are greatly indebted to the Calco Chemical Division of the American Cyanamid Co. for gifts of chemicals, the Sir Dorabji Tata Trust for a scholarship (to S.K.M.), and Dr. A. Sreenivasan and Mr. M. S. Pavgi for co-operation in carrying out the bacteriostatic tests.

References

1. Dhingra, Uppal and Venkataraman .. *J. Soc. Dyers Col.*, 1937, **53**, 91; *et sequa.*
2. Domagk .. *Deutsch. med. Wochs.*, 1935, **61**, 289;
   Miller and Baker .. *Science*, 1940, **91**, 624;
   Baker, *et al.*, .. *J. Expl., med.*, 1941, **73**, 249.
3. Gershenfeld *et al.* .. *Amer. J. Pharm.*, 1941, **113**, 306; 1946, **18**, 228.
4. Crossley, *et al.* .. *J. Amer. Chem. Soc.*, 1939, **61**, 2950.
   Rajagopalan .. *Curr. Sci.*, 1942, **10**, 394.
5. Forster, *et. al.* .. *J. Soc. Chem. Ind.*, **1924**, **43**, 165 ; *et sequa.*
6. Smyth and Carpenter .. *Science*, 1938, **87**, 350.
7. Ebert .. U. S. P. 2,182,075.
8. Mossini .. *Boll. Chim. Farm.*, 1939, **78**, 429.
9. Woelm .. B. P. 490,350.
10. ———— .. F. P. 831,300.
11. Kertess .. *Text. Manuf.*, 1934, **60**, 336,
    Kling and Puschell, .. *Milliand, Textilber*, 1934, **15**, 21; *Amer.*
    Shiraeff .. *Dyestuff, Rep.*, 1947, **36**, 313.
12. Shirolkar and Venkataraman .. *J. Soc. Dyers Col.*, 1941, **57**, 41;
    Daruwala *et al.* .. *J. Ind. Chem. Soc.*, Ind. Ed., 1944, **60**, 24.
13. Woods and Fildes .. *Lancet*, 1940, **238**, I, 955.
14. Bell and Roblin .. *J. Amer. Chem. Soc.*, 1942, **64**, 2907.
15. Kumler and Daniels .. *Ibid.*, 1943, **65**, 2190.
    Bordwell and Klotz .. *Ibid.*, 1944, **66**, 660.
    Bell, *et al.* .. *Ibid.*, 847.
16. Klotz .. *Ibid.*, 464.
    Klotz and Gutman .. *Ibid.*, 1945, **67**, 558.
17. ——— and Gruen .. *Ibid.*, 843.
18. Fuller .. *Manuf. Chemist*, 1947, **18**, 416.
19. Cohen and Edsall .. *Proteins, Amino Acids and Peptides* (Reinhold), 40, 455, 590, 599.
20. Roblin .. *Chem. Rev.*, 1946, **38**, 358.
21. Muir *et al.* .. *J. Bact.*, 1942, **44**, 95.