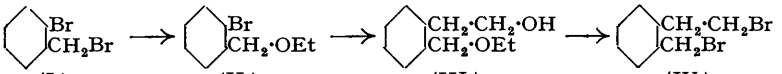
160. *The Synthetic Application of* o-β-*Bromoethylbenzyl Bromide. Part I. Sulphanilamide Derivatives of* 1 : 2 : 3 : 4-*Tetrahydro*isoquinoline.

By Frederick G. Holliman and Frederick G. Mann.

A method for preparing *o*-β-bromoethylbenzyl bromide readily in good yield is described. A number of sulphanilamide derivatives containing the 2-(1 : 2 : 3 : 4-tetrahydro*iso*quinolyl) group, and also certain sulphanilic hydrazide derivatives, have been prepared and their bactericidal properties determined.

o-β-Bromoethylbenzyl bromide (IV) has obviously great synthetic value for the preparation of *N*-substituted 1 : 2 : 3 : 4-tetrahydro*iso*quinolines and of related compounds in which the nitrogen is replaced by other elements. Its past use has been severely limited, however, by its laborious preparation. Von Braun and Zobel (*Ber.*, **1923**, **56**, 2142) have described a synthesis of the bromide from *o*-toluidine which involves eleven stages : if the highest yield by modern methods for the conversion of *o*-toluidine into *o*-cyanobenzyl bromide is taken (65%), and von Braun and Zobel's optimum yields for the remaining nine stages then considered, the over-all yield of the *o*-β-bromoethylbenzyl bromide from *o*-toluidine is 3·5% of the theoretical. The practical value of this synthesis is clearly very small.

We have worked out a synthesis from *o*-toluidine which involves five stages and gives an over-all yield of 8%. *o*-Toluidine is converted into *o*-bromotoluene and the latter into *o*-bromobenzyl bromide (I) by the method of Kenner and Wilson (J., 1927, 1110). Treatment with sodium ethoxide gives o-*bromobenzyl ethyl ether* (II). The conversion of the latter into a Grignard reagent at first gave great difficulty : ultimately, by



(I.) (II.) (III.) (IV.)

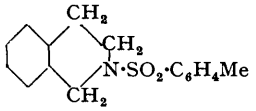
using specially activated magnesium and diluting the bromo-ether (II) with ethyl bromide (Grignard's " entrainment method," *Compt. rend.*, **1934**, **198**, 625), the reagent was satisfactorily prepared, and when treated with ethylene oxide gave *o*-β-hydroxyethylbenzyl ethyl ether (III), the penultimate stage in von Braun and Zobel's synthesis. These authors converted this ether (III) into *o*-β-bromoethylbenzyl bromide (IV) by the action of hydrobromic acid, purifying the final product by distillation : we find that the use of hydrogen bromide in acetic acid gives a high yield of the pure crystalline dibromide (IV), and the distillation of this intensely lachrymatory substance is thus avoided.

Our preparative method has now rendered the dibromide (IV) freely available and we have consequently investigated its synthetic application in several directions.

In view of the frequent occurrence of the reduced *iso*quinoline system in alkaloids possessing marked physiological activity, it was of interest to examine first the therapeutic effect of introducing this group into the sulphanilamide molecule.

For a preliminary testing of the method to be employed, an intimate mixture of equimolecular quantities of the dibromide (IV) and of *p*-toluenesulphonamide, together with excess of potassium carbonate, was heated at 160—170° for 30 minutes : the reaction proceeded smoothly, and the product readily yielded 2-p-*toluenesulphonyl*-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (V). The same product was also obtained when the above mixture was boiled in alcoholic solution for 5 hours. Since this compound lacks the characteristic

p-amino-group of the sulphanilamide molecule, it was not, of course, expected to possess marked anti-bacterial action.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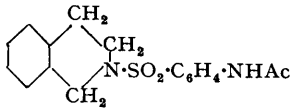
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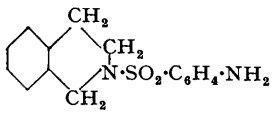
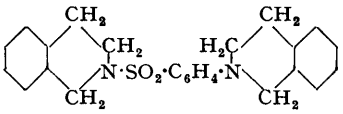
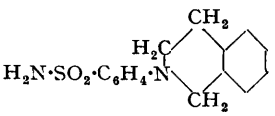

(VII.)

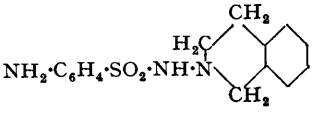
When, however, a similar mixture of the dibromide (IV), *p*-acetamidobenzenesulphonamide and potassium carbonate was boiled in alcohol, 2-p-*acetamidobenzenesulphonyl*-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (VI) was obtained, and this compound was readily hydrolysed by hot dilute hydrochloric acid to the corresponding 2-p-*amino*-compound (VII). The latter could also be prepared by the condensation of equimolecular quantities of the dibromide (IV) and of sulphanilamide, but this method entailed the separation of the required *p*-amino-compound (VII) from the 2-[p-(2′-1′ : 2′ : 3′ : 4′-*tetrahydro*isoquinolyl)*benzenesulphonyl*]-1 : 2 : 3 : 4-*tetrahydro*-isoquinoline (VIII) which was always formed as a by-product: the method involving the hydrolysis of the acetamido-compound is therefore preferable. The di*iso*quinolyl compound (VIII) itself could of course be more readily prepared by the direct condensation of two molecules of the dibromide (IV) with one of sulphanilamide.

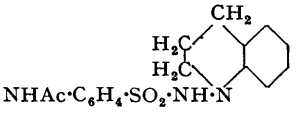
(VIII.) 


(IX.)

The preparation of p-(2-1 : 2 : 3 : 4-*tetrahydro*isoquinolyl)*benzenesulphonamide* (IX), isomeric with (VII), gave much greater difficulty than that of the previous derivatives. The condensation of the dibromide (IV) and sodium sulphanilate, followed by acidification, readily gave p-(2-1 : 2 : 3 : 4-*tetrahydro*isoquinolyl)*benzene-sulphonic acid*, but the conversion of this acid into its *amide* (IX), although attempted under various conditions, was never satisfactory: the action of phosphorus pentachloride, followed by aqueous ammonia, furnished the pure amide, but only if small quantities were used, and even then in low yield. Consequently sufficient of this amide (IX) for adequate therapeutic tests was not obtained: this compound would probably be without anti-bacterial action, however, as almost all *p*-(disubstituted-amino)benzenesulphonamides are inert in this respect.

Two syntheses of *p*-aminobenzenesulphon-2-(1 : 2 : 3 : 4-tetrahydro*iso*quinolyl)amide (X) have been unsuccessfully attempted. An equimolecular mixture of the dibromo-compound (IV) and of *p*-acetamido-benzenesulphonhydrazide (Curtius and Stoll, *J. pr. Chem.*, **1926**, **112**, 125) underwent condensation in the presence of potassium carbonate, but the ultimate product was a glass from which no crystalline product could be obtained. Alternatively, many attempts were made to reduce 2-nitroso-1 : 2 : 3 : 4-tetrahydro*iso*-quinoline to the 2-amino-compound, in order to condense the latter with *p*-acetamidobenzenesulphonyl chloride, but all such attempts failed in spite of a variety of conditions employed.

(X.) 


(XI.)

In an attempt to prepare the quinolyl isomeride of (X), *p*-acetamidobenzenesulphonyl chloride was condensed with 1-amino-1 : 2 : 3 : 4-tetrahydroquinoline to give p-*acetamidobenzenesulphon*-1-(1 : 2 : 3 : 4-*tetra-hydroquinolyl*)*amide* (XI), but all attempts to hydrolyse the acetyl group from this compound caused extensive decomposition.

Since the therapeutic properties of the sulphanilic hydrazides had not been recorded, the preparation of *p*-aminobenzenesulphonhydrazide (XII) by the hydrolysis of the *p*-acetamido-compound (Curtius and Stoll, *loc. cit.*) was first considerably improved. The *p*-acetamido-compound was then condensed with *p*-acetamido-benzenesulphonyl chloride in the presence of pyridine to give s.-*di*-p-*acetamidobenzenesulphonhydrazide* (XIII).

$NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH \cdot NH_2$ (XII.)

$[NHAc \cdot C_6H_4 \cdot SO_2 \cdot NH^-]_2$ (XIII.)

$[NH_2 \cdot C_6H_4 \cdot SO_2 \cdot NH^-]_2$ (XIV.)

The constitution of this compound was shown by the fact that it was readily soluble in alkalis and insoluble in acids: the *as.*-isomeride, had it been formed, would have had these solubilities reversed. Hydrolysis then gave the s.-*di*-p-*amino*-compound (XIV) as a stable monohydrate.

Tests kindly carried out by Dr. G. Brownlee at the Wellcome Physiological Research Laboratories show that compounds (V), (VI), (VII), and (VIII) are almost without action on Staphylococcus and Streptococcus in a meat extract broth at 37°. The compounds (XI), (XII), and (XIV) were similarly tested with Staphylo-coccus, Streptococcus, *B. coli* and *B. typhosus*, and showed some bacteriostatic activity against the first: all three compounds, however, were completely inactive against Staphylococcus infection in mice.

Experimental.

Pure *o*-bromotoluene, prepared as described in *Organic Syntheses*, 1929, **9**, 22, an aqueous solution of sodium nitrite being used instead of the anhydrous salt, was obtained in 33% yield. In the bromination to *o*-bromobenzyl bromide, 85% of the theoretical quantity of bromine was used instead of 75% as recommended by Kenner and Wilson (*loc. cit.*): allowing for recovered unchanged material, the yield of pure *o*-bromobenzyl bromide (I) was 80% of the theoretical. This yield was obtained only if the bromine was added steadily during 3 hours without agitation of the reaction mixture.

o-Bromobenzyl Ethyl Ether (II).—*o*-Bromobenzyl bromide (190 g.) was added to a solution of sodium ethoxide prepared from sodium (20 g., 1·15 atoms) and absolute alcohol (450 c.c.) and chilled in ice-water. The mixture was refluxed until it had boiled for 15 minutes and developed an orange colour. Precipitated sodium bromide was removed from the hot solution, the latter concentrated, and the residual oil poured into water and repeatedly extracted with ether. Fractional distillation of the dried extract gave *o-bromobenzyl ethyl ether* as a colourless liquid, b. p. 119—120°/18 mm. (Found: C, 50·0; H, 5·0. $C_9H_{11}OBr$ requires C, 50·2; H, 5·1%): yield, 160 g., 98% of the theoretical.

o-β-Hydroxyethylbenzyl Ethyl Ether (III).—A mixture of fine magnesium filings (10 g.) and powdered iodine (5 g.) was triturated and then heated with continuous agitation in a long-necked flask until no more iodine vapour was evolved. The hot product was quickly and carefully (owing to its pyrophoric nature) transferred to a clean dry flask, which was corked until the product was cold. This activated magnesium was prepared immediately before use.

A solution of *o*-bromobenzyl ethyl ether (100 g.; 0·46 mol.) and ethyl bromide (17·4 g.; 0·16 mol.) in ether (200 c.c.) was added to the magnesium (24 g.; 1 atom) under ether (50 c.c.) at such a rate that the reaction, which started rapidly, kept the solvent gently boiling under reflux. When the addition was complete, boiling by external heating was maintained for 4 hours. The Grignard reagent was then chilled in ice–salt and continuously stirred while a solution of ethylene oxide (60 g.; 1·36 mols.) in ether (400 c.c.) was added in small portions (10 c.c.) during 4 hours. The product was set aside overnight and then again chilled and stirred while dilute sulphuric acid (600 c.c., 1 vol. of acid: 9 vols. of water) was slowly added. The product was filtered, and the ethereal solution washed in turn with water and sodium carbonate solution, dried (potassium carbonate), and fractionally distilled. The ether (III) was obtained having b. p. 152—155°/13 mm. (Found: C, 72·5; H, 8·7. Calc. for $C_{11}H_{16}O_2$: C, 73·3; H, 8·9%): yield, 29 g., 35% of the theoretical. Von Braun and Zobel (*loc. cit.*) give b. p. 149—152°/12 mm. This product, which could not be further purified by distillation, was used without further treatment for the preparation of the bromide (IV).

The fractional distillation of the above crude ether (III) always gave a lower fraction, b. p. 58°/14 mm., which on refractionation at atmospheric pressure gave a main fraction, b. p. 146—147°. Although analysis showed that the latter fraction was still a mixture, its chief component was ethylene bromohydrin, since with phenyl *iso*cyanate it readily gave phenyl-β-bromourethane, m. p. 74—75°, unchanged by admixture with a sample, m. p. 74—75°, prepared from pure ethylene bromohydrin (Found: C, 44·1; H, 4·2; N, 5·8. Calc. for $C_9H_{10}O_2NBr$: C, 44·3; H, 4·1; N, 5·7%).

o-β-Bromoethylbenzyl Bromide (IV).—A mixture of the hydroxy-ether (III) (22 g.) and a solution of hydrogen bromide in acetic acid (90 g., containing 50 g. of HBr/100 c.c. of solution) in a stoppered bottle was immersed in a boiling water-bath for 24 hours. The cold product was poured into water (*ca.* 1 l.), the heavy dark oil, which usually solidified, extracted with ether, and the extract washed in turn with aqueous sodium carbonate and water, and dried. Distillation of the ether left the oily dibromide, which readily solidified: yield, 34 g., 90%. Recrystallisation from light petroleum (b. p. 40—60°) (charcoal) gave the dibromide in colourless needles, m. p. 51—53°, and this product was used without further treatment for subsequent synthetic work. Further recrystallisation from alcohol gave the pure material, m. p. 53° (Found: C, 38·55; H, 3·8. Calc. for $C_9H_{10}Br_2$: C, 38·9; H, 3·6%): von Braun and Zobel also give m. p. 53°.

2-p-*Toluenesulphonyl*-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (V).—An intimate powdered mixture of the dibromide (IV) (2·8 g.), *p*-toluenesulphonamide (1·7 g.; 1 mol.), and anhydrous potassium carbonate (2·1 g.; 1·5 mols.) was heated in an oil-bath to 160° and the temperature then kept at 160—170° for 30 minutes: the reaction started at about 90° with carbon dioxide evolution. The cold hard mass was pulverised and extracted with boiling alcohol, which after filtration and cooling deposited the p-*toluenesulphonyl* derivative (V) in colourless needles (2·5 g.), m. p. 141—142°, increased to 142° by one recrystallisation from alcohol (Found: C, 66·8; H, 6·05; N, 4·8. $C_{16}H_{17}O_2NS$ requires C, 66·9; H, 5·9; N, 4·9%).

2-p-*Acetamidobenzenesulphonyl*-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (VI).—A mixture of the dibromide (IV) (2·8 g.), *p*-acetamidobenzenesulphonamide (2·1 g.; 1 mol.), anhydrous potassium carbonate (4·1 g.; 3 mols.), and alcohol (20 c.c.) was refluxed for 5 hours. The liquid was filtered hot, and the residue extracted with boiling alcohol (20 c.c.). The combined alcoholic solutions deposited the colourless crystalline p-*acetamido*-derivative (VI), m. p. 170—174° (3·3 g.): two recrystallisations from alcohol, followed by heating in a vacuum at 100°, gave the pure compound, m. p. 175—176° (Found: C, 61·6; H, 5·9; N, 8·2. $C_{17}H_{18}O_3N_2S$ requires C, 61·8; H, 5·45; N, 8·5%).

2-p-*Aminobenzenesulphonyl*-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (VII).—(A) *By hydrolysis of the acetyl derivative.* A mixture of the above acetyl derivative (VI) (6 g.) and dilute hydrochloric acid (90 c.c., 1 vol. of conc. acid: 3 vols. of water) was refluxed for 1·5 hours. After cooling, the crude solid was collected, boiled with 8% sodium carbonate solution (120 c.c.), again collected, washed with water, dried, and recrystallised from benzene containing a small quantity of alcohol. The p-*aminobenzene* derivative (VII) was thus obtained as colourless crystals, which, after heating in a vacuum at 100° to eliminate traces of solvent, had m. p. 174° with softening at 172° (Found: C, 62·4; H, 5·5; N, 9·7. $C_{15}H_{16}O_2N_2S$ requires C, 62·5; H, 5·55; N, 9·7%): a mixture with unchanged acetyl compound had m. p. 164—170°.

(B) *By direct condensation.* A mixture of the dibromide (IV) (2·8 g.), sulphanilamide (1·7 g.; 1 mol.), potassium carbonate (4·1 g.; 3 mols.), and alcohol (20 c.c.) was refluxed for 5 hours and filtered, and the residue further extracted with hot alcohol. The united alcoholic solutions on cooling deposited crystals, which were purified precisely as in (A) above, except that three recrystallisations were now necessary. The final product had m. p. identical with that in (A), both mixed and unmixed.

The insoluble residue from the above alcoholic extraction, when washed with water to remove inorganic material, furnished the di*iso*quinolyl compound (VIII) described below, being identified (after purification) by analysis (Found: N, 7·1%) and mixed m. p. determination.

2-[p-(2′-1′ : 2′ : 3′ : 4′-*Tetrahydro*isoquinolyl)*benzenesulphonyl*]-1 : 2 : 3 : 4-*tetrahydro*isoquinoline (VIII).—The condensation described in (B) above was repeated, only half the sulphanilamide (0·5 mol.) being used. The solid residue insoluble in boiling alcohol was washed thoroughly with water and recrystallised from alcoholic acetone, the *compound* (VIII) being obtained in colourless crystals, m. p. 153—156° (Found: C, 71·4; H, 6·0; N, 7·0. $C_{24}H_{24}O_2N_2S$ requires C, 71·3; H, 5·9; N, 6·9%). The m. p. of this compound showed inexplicable variations. For instance, the above pure sample, after being kept in a vacuum desiccator for 1 week, softened at 140° and had m. p. 142—144°; another pure sample, similarly kept for 3 weeks, melted at 141—142° to an opaque liquid which became clear at 153—154°, although the composition was unaltered (Found: N, 6·9%). The pure material, after recrystallisation from benzene–*cyclo*hexane, softened at 144° and melted at 149°; after recrystallisation from carbon tetrachloride, it softened at 153° and melted at 157—157·5°. All m. p.'s were determined under approximately identical conditions.

p-(2-1 : 2 : 3 : 4-*Tetrahydroisoquinolyl*)*benzenesulphonamide* (IX).—A mixture of the dibromide (IV) (2·8 g.), sodium sulphanilate (1·9 g.; 1 mol.), anhydrous sodium carbonate (4·3 g.; 4 mols.), and alcohol (20 c.c.) was boiled for 6 hours and cooled. The solid residue was twice recrystallised from hot water : the colourless crystals of the sodium sulphonate corresponding to (IX) thus obtained were redissolved in hot water; the solution, when acidified with hydrochloric acid, deposited the crystalline p-(2-1 : 2 : 3 : 4-*tetrahydroisoquinolyl*)*benzenesulphonic acid*, which was washed with a small quantity of boiling water and twice recrystallised from much boiling water, in which it was only slightly soluble : it was thus obtained as a microcrystalline *hemihydrate*, m. p. 236—237° (efferv.) (Found : C, 60·3; H, 5·5; N, 4·6. $C_{15}H_{15}O_3NS,\frac{1}{2}H_2O$ requires C, 60·4; H, 5·4; N, 4·7%).

An intimate powdered mixture of the above acid (1·1 g.) and phosphorus pentachloride (0·8 g.; 1·04 mol.) was heated at 120° for 15 minutes, hydrogen chloride being evolved. The cold brown hard product was powdered, added to aqueous ammonia (*d* 0·880, 30 c.c.), and the mixture heated on a boiling water-bath for 30 minutes. The brown powder so obtained was collected, washed with water, and dried : it was freely soluble in cold dilute sodium hydroxide solution or hydrochloric acid, and was reprecipitated from each solution on neutralisation. This powder was dissolved in hot ethyl alcohol, a small quantity of brown microcrystalline material which separated on cooling was removed, and the filtrate taken to dryness. The residue, twice recrystallised from methyl alcohol (charcoal), gave the *sulphonamide* (IX) in pale brown needles, which softened at 161°, melted at 163°, resolidified and remelted at 182—184° : even after heating in a vacuum at 100° for 3 hours, this behaviour was unchanged, and it was therefore not due to solvent of crystallisation (Found for the unheated material : C, 62·6; H, 5·8; N, 9·6. $C_{15}H_{16}O_2N_2S$ requires C, 62·5; H, 5·55; N, 9·7%).

Attempted Preparation of the Amide (X).—(i) A mixture of *p*-acetamidobenzenesulphonhydrazide (2·3 g.), the dibromide (IV) (2·8 g.; 1 mol.), potassium carbonate (4 g.; 2·9 mols.), and absolute alcohol (50 c.c.) was refluxed for 8 hours, and then filtered hot from the entirely inorganic residue. The filtrate was evaporated on a water-bath, giving a very viscous oil which set to a glass on cooling. The glass contained nitrogen and sulphur, but no halogen : it did not crystallise even after several months, and no crystalline constituent or derivative could be isolated.

(ii) 2-Nitroso-1 : 2 : 3 : 4-tetrahydro*iso*quinoline, m. p. 51—53°, was prepared by Bamberger and Dieckmann's method (*Ber.*, 1893, **26**, 1211). The reduction of this compound was attempted (*a*) in alcoholic acetic acid with zinc dust, (*b*) in neutral alcoholic solution with aluminium amalgam (Mann and Pope, *Proc. Roy. Soc.*, 1925, *A*, **107**, 86) : although the temperature and concentrations were varied over wide limits, complete reduction to 1 : 2 : 3 : 4-tetrahydro-*iso*quinoline always occurred.

p-*Acetamidobenzenesulphon*-1-(1 : 2 : 3 : 4-*tetrahydroquinolyl*)*amide* (XI).—(*a*) 1 : 2 : 3 : 4-Tetrahydroquinoline was prepared by Oldham and Johns's method (*J. Amer. Chem. Soc.*, 1939, **61**, 3289), b. p. 121°/13 mm.; hydrochloride, m. p. 180—182°. (*b*) No specific directions for the next two stages have been published. A solution of sodium nitrite (10·7 g.) in water (50 c.c.) was slowly added with stirring and external cooling to a solution of tetrahydroquinoline (20 g.; 1 mol.) in concentrated hydrochloric acid (38 c.c.) diluted with water (60 c.c.) and ice (50 g.), the temperature being kept below 10° throughout. One hour after the addition, the yellow oily 1-nitroso-1 : 2 : 3 : 4-tetrahydroquinoline (22 g., 92%) was extracted with ether, dried (potassium carbonate), and, after removal of the ether, reduced without further purification (cf. Ziegler, *Ber.*, 1888, **21**, 862). (*c*) The nitroso-compound (22 g.), dissolved in a mixture of acetic acid (45 c.c.), water (45 c.c.), and alcohol (70 c.c.), was slowly added to a vigorously stirred suspension of zinc dust (100 g.) in 90% alcohol (130 c.c.) maintained at 60—75°. When reduction was complete, the filtered solution was evaporated under reduced pressure to remove alcohol, treated with 30% caustic soda solution until the zinc hydroxide redissolved, and then extracted with ether. The ethereal solution was shaken with dilute sulphuric acid (50 c.c., 1 vol. of acid : 9 vols. of water), and the precipitated 1-amino-1 : 2 : 3 : 4-tetrahydroquinoline sulphate collected and recrystallised from water : yield, 11 g. Treatment with alkali gave the 1-amino-base, m. p. 53—55° (cf. Hofmann and Koenigs, *Ber.*, 1883, **16**, 730; Barger and Dyer, *J. Amer. Chem. Soc.*, 1938, **60**, 2414). (*d*) The aminotetrahydroquinoline sulphate (4·3 g.) was added to a vigorously stirred solution of caustic soda (2·8 g.; 7 mols.) in water (80 c.c) maintained at 60°, and *p*-acetamidobenzenesulphonyl chloride (5·1 g.; 2·6 mols.) then slowly added, the stirring being continued for 1 hour. The solid which had separated was collected, and purified by dissolution in warm aqueous 10% caustic soda, filtration, and reprecipitation with hydrochloric acid : a second crop was obtained by acidifying the original mother-liquor. The united crops were extracted with sodium carbonate solution, and the crude insoluble product (3 g.), after recrystallisation from much alcohol, gave p-*acetamidobenzenesulphon*-1-(1 : 2 : 3 : 4-*tetrahydroquinolyl*)*amide* in colourless crystals, m. p. 203° (decomp.) (Found : C, 59·1; H, 5·8; N, 12·3. $C_{17}H_{19}O_3N_3S$ requires C, 59·1; H, 5·6; N, 12·2%).

The attempted hydrolysis of this compound, hydrochloric acid or caustic soda being used with a wide variety of concentration and time of heating, always gave either unchanged material or extensive decomposition, and the required *p*-amino-compound could not be isolated.

p-*Aminobenzenesulphonhydrazide* (XII).—The following preparative method was employed, as Curtius and Stoll's directions (*loc. cit.*) always gave unchanged material. A smooth paste of powdered *p*-acetamidobenzenesulphonhydrazide (5 g.) and concentrated hydrochloric acid (8 c.c.) was stirred mechanically while heated on a boiling water-bath. Five minutes' heating gave a clear solution, which after a further 7 minutes was cooled, neutralised with 3% caustic soda solution, and kept at 0° for 1 hour. The precipitated material was twice recrystallised from 50% aqueous alcohol, and the *p*-amino-compound (XII) obtained as colourless crystals (1·2 g., 29%), m. p. 132° (efferv.) (Found : C, 38·5; H, 4·8; N, 22·7. Calc. for $C_6H_9O_2N_3S$: C, 38·5; H, 4·8; N, 22·4%).

s.-*Di*-p-*acetamidobenzenesulphonhydrazide* (XIII).—*p*-Acetamidobenzenesulphonyl chloride (4·6 g.) was added to a solution of the *p*-acetamido-hydrazide (4·6 g.; 1 mol.) in dry pyridine (20 c.c.), and the solution heated at 100° for 30 minutes, cooled, and poured into water (400 c.c.). The white microcrystalline precipitate was collected, washed with water, and, since it was insoluble in all the usual organic liquids, purified by dissolution in N-sodium hydroxide (50 c.c.), filtration, and reprecipitation with dilute hydrochloric acid. The *di*-p-*acetamido*-compound (XIII) (3·75 g., 44%) so obtained, when washed with boiling water and alcohol and dried, was unaffected by heating to 300° (Found : C, 45·1; H, 4·5; N, 13·1. $C_{16}H_{18}O_6N_4S_2$ requires C, 45·1; H, 4·2; N, 13·1%).

s.-*Di*-p-*aminobenzenesulphonhydrazide* (XIV).—A mixture of the acetamido-compound (XIII) (4 g.), concentrated hydrochloric acid (15 c.c.), and water (30 c.c.) was refluxed for 4 hours. After a further short boiling with charcoal, filtration gave a colourless solution. This was treated with a solution of caustic soda (5 g.) in water (15 c.c.) and then with saturated aqueous sodium carbonate until alkaline. The precipitated *di*-p-*amino*-compound (XIV) (2·3 g., 72%) was collected, washed with water, and recrystallised from 50% aqueous alcohol, from which it separated as a stable crystalline *monohydrate* : the latter lost water slowly in a vacuum over sulphuric acid, and readily when heated at 135—140°/15 mm., but the anhydrous material rapidly re-formed the hydrate on exposure to air (Found for the monohydrate : C, 39·8; H, 4·6; N, 15·4; S, 18·1; H_2O, 5·15. $C_{12}H_{14}O_4N_2S_2,H_2O$ requires C, 40·0; H, 4·4; N, 15·55; S, 17·8; H_2O, 5·0%. Found for the anhydrous substance : N, 16·1. $C_{12}H_{14}O_4N_4S_2$ requires N, 16·4%). Both the anhydrous material and the hydrate darkened at 190° and melted at 203° (decomp.).

Dr. Brownlee reports : The compounds (XI), (XII), and (XIV) were tested *in vitro* at various concentrations against

constant inocula of Streptococcus, Staphylococcus, *B. coli* and *B. typhosus* (1000—2000 per c.c.). Owing to their low solubility, the highest concentrations obtainable were 1/12,500 for (XI) and 1/2500 for (XII) and (XIV). All three compounds showed bacteriostatic activity against Staphylococcus, arresting growth for 18 hours—after this period the culture developed when (XI) was at its highest concentration (1/12,500) and when (XII) and (XIV) were at a concentration of 1/5000, but showed no visible growth even after 48 hours when (XII) and (XIV) were at their highest concentration (1/2500).

When tested against Staphylococcus infection in mice, using doses of 10 mg. of (XI) and (XIV) per 20 g. of mouse, and of 5 mg. of (XII) per 20 g., all three compounds were completely inactive.

The compounds were tested for toxicity, and compound (XII), as was to be expected of a hydrazide, was lethal at a dose of 100 mg. per 20 g. of mouse, but (XI) and (XIV) were not.

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