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# Studies on Sulphatases

## 10. THE ISOLATION AND CHARACTERIZATION OF BIOSYNTHETIC ARYLSULPHATES*

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Most phenols are partially excreted as the corresponding sulphate esters although, owing to the great solubility of the compounds and their sodium and potassium salts, few arylsulphates have been isolated from urine. The less soluble *p*-toluidine (Barton & Young, 1943) and *p*-bromoaniline (Laughland & Young, 1944) arylsulphate salts have been isolated after some preliminary treatment of the urine (Berenbom & Young, 1951) but isolation in this way is not very satisfactory when the arylsulphates are present in small amounts. Other naturally occurring sulphate esters are difficult to isolate from natural sources. They are usually precipitated from aqueous solution by organic solvents.

Egami (1939) noted that trypaflavin (2:8-diamino-10-methylacridinium chloride), safranine (2:8-diamino-10-phenylphenazonium chloride) and rivanol (5:8-diamino-3-ethoxyacridine) gave insoluble salts with a number of different sulphate esters and suggested that these bases might be used to separate naturally occurring sulphates. In the present work a number of aminoacridines and related compounds have been examined as precipitating agents for organic sulphates, particularly arylsulphates. Many of these bases gave insoluble salts with sulphate esters and the most suitable, 5-aminoacridine, was used to isolate the sulphated phenols arising from the metabolism of chlorobenzene, chlorophenol and 4-chlorocatechol. A preliminary account of this work has already appeared (Dodgson, Rose & Spencer, 1954).

* Part 9: Dodgson *et al.* (1955).

### MATERIALS

Euflavin (2:8-diamino-10-methylacridinium chloride), safranine, 5-aminoacridine hydrochloride, 2- and 3-aminoquinolines and 2-aminopyridine were commercial samples. 6- and 8-Aminoquinolines were obtained by reduction of the corresponding nitroquinolines by the methods of Linsker & Evans (1946) and Dikshoorn (1929), respectively. 5- and 7-Aminoquinolines were prepared from the corresponding 5- and 7-nitroquinolines (Bradford, Elliott & Rowe, 1947) by reduction, using the methods of Dikshoorn (1929) and Linsker & Evans (1946), respectively. 4-Amino-2-methylquinoline: 2-methylcinchonamide was obtained from isatin by the method of Bayer (1916) and was subsequently converted into 4-amino-2-methylquinoline according to the directions of Lions & Ritchie (1941). Arylsulphates were prepared by the method of Burkhardt & Lapworth (1926).

### EXPERIMENTAL AND RESULTS

#### *The precipitation of arylsulphates by aminoacridines and safranine*

Three arylsulphates, phenyl sulphate (PS), *p*-nitrophenyl sulphate (NPS) and *p*-acetylphenyl sulphate (APS) were selected for study. Molar proportions (usually 0·002 M) of the arylsulphate (potassium salt) and the precipitating reagent

Table 1. *Properties of the euflavin, 5-aminoacridine and safranine salts of phenyl, p-acetylphenyl and p-nitrophenyl sulphates*

| Base | Aryl-sulphate | Colour | Properties of salts: Solubility in water (mg./100 ml.) at 20° | at 4° | % $SO_4$ Calc. | % $SO_4$ Found | Water of crystallization (calc.) (mol.) | Recrystallization medium |
|---|---|---|---|---|---|---|---|---|
| Euflavin | PS | Orange-red | 50 | 42 | 8·1 | 8·0 | 0 | Water |
| | APS | Orange-red | 89 | 58 | 7·3 | 7·3 | 0 | 75% (v/v) ethanol |
| | NPS | Orange | 256 | 56 | 7·3 | 7·2 | 0 | Ethanol |
| 5-Aminoacridine hydrochloride | PS | Yellow | 121 | 65 | 8·7 | 8·6 | 0 | Water |
| | APS | Yellow | 28 | 6 | 8·6 | 7·6 | ½ | Water |
| | NPS | Yellow | 72 | 42 | 7·6 | 7·6 | ½ | Water |
| Safranine | PS | Brown | 30 | 30 | 6·5 | 6·3 | 2 | Water + trace of ethanol |
| | APS | Red | 3 | 2 | 5·9 | 5·9 | 2 | Water + trace of ethanol |
| | NPS | Red-brown | 7 | 4 | 5·9 | 6·0 | 2 | Water + trace of ethanol |

Table 2. *Absorption maxima and molecular extinction coefficients of potassium phenyl sulphate, euflavin phenyl sulphate, euflavin, 5-aminoacridine and safranine*

Absorption spectra were determined in water using the Hilger Uvispec.

| Compound | $\lambda_{max.}$ (mμ.) | $\epsilon_{262}$ | $\epsilon_{454}$ |
|---|---|---|---|
| Euflavin phenyl sulphate | 262, 454 | 59 800 | 42 300 |
| Euflavin | 262, 454 | 49 200 | 42 900 |
| Phenyl sulphate | 251 | 10 800 | 0 |
| | | $\epsilon_{max.}$ | |
| 5-Aminoacridine hydrochloride | 383 | 6 150 | |
| | 402 | 8 800 | |
| Safranine | 424 | 7 340 | |
| | 520 | 38 600 | |

(euflavin, safranine or 5-aminoacridine hydrochloride) were dissolved in 10 and 20 ml. water, respectively, and mixed at room temp. In all cases microcrystalline precipitates were formed which were separated by filtration, washed well with ice-cold water and recrystallized from water, ethanol or aqueous ethanol. The properties of the salts (which had ill-defined melting points) are described in Table 1.

*Absorption spectra and solubilities of the salts.* The ionic nature of the reaction between arylsulphate and base, which was noted by Egami (1939), was confirmed by the absorption spectra of the salts and their components since the spectrum of euflavin phenyl sulphate was the additive spectrum of euflavin and the arylsulphate (Table 2).

The absorption spectra of euflavin, 5-aminoacridine and safranine (Table 2) showed maxima at wavelengths 454, 402 and 520 mμ., respectively, where PS, APS and NPS showed no absorption. At these wavelengths the molar extinction coefficients of the arylsulphate salts of the bases are the same as those of the respective bases alone. It was thus possible to determine the solubility of the salts (Table 1) by spectrophotometric measurement of the concentration of each base present in saturated solution at 20° and 4°.

### *The precipitation of arylsulphates by aminoquinolines*

A number of aminoquinolines gave insoluble salts with PS, APS and NPS. Equimolar proportions (usually mol.wt./600) of arylsulphate and the aminoquinoline hydrochloride, dissolved in 5 and 10 ml. water, respectively, were mixed, cooled to 0° and frequently stirred and scratched. The salts were separated, washed with a little ice-cold water and recrystallized thrice from water. Table 3 shows that crystalline salts were not obtained in all cases. PS showed less tendency than APS or NPS to form crystalline salts and tended to form crystalline derivatives with the more basic aminoquinolines only (cf. Albert & Goldacre, 1944). The aminoquinoline salts were more soluble than those of the aminoacridines.

### *Preparation of potassium arylsulphates from the corresponding 5-aminoacridine salts*

Of the bases examined, 5-aminoacridine hydrochloride was considered the most suitable for general use. Safranine gave less soluble salts but they were more difficult to crystallize. Although arylsulphates may readily be obtained as the insoluble 5-aminoacridine salts it may sometimes be necessary to convert these salts into more soluble ones for further study. Two methods were used for the preparation of the soluble potassium derivatives.

(i) *Treatment with potassium hydroxide.* 5-Aminoacridine *p*-nitrophenyl sulphate (2·48 g.) was suspended in 200 ml. water at 60°. A solution of KOH (0·34 g. in 5 ml. water) was added with stirring and the mixture heated to boiling. After cooling at 0° the liberated 5-aminoacridine was filtered off and the filtrate concentrated *in vacuo* at 35° until about 10 ml. remained. After addition of a few drops of ethanol followed by cooling at 0°, 1·22 g. of potassium *p*-nitrophenyl sulphate separated out (yield, 79%). Repetition of this procedure with 5-aminoacridine *p*-acetylphenyl sulphate gave a 78% yield of the corresponding potassium salt.

Table 3. *The solubilities of the aminoquinoline salts of arylsulphates*

Solubilities were determined by acid hydrolysis of saturated solutions of the salts and subsequent turbidimetric estimation of the liberated sulphate (Nalefski & Takano, 1950).

| Aminoquinoline | Aryl-sulphate | Colour of crystals | Solubility in water (mg./100 ml.) At 20° | At 4° |
|---|---|---|---|---|
| 2-Aminoquinoline | PS | Yellow | 1150 | 640 |
| | APS | Colourless | 540 | 328 |
| | NPS | Yellow | 540 | 317 |
| 3-Aminoquinoline | PS | Oil | — | — |
| | APS | Yellow | 1900 | 850 |
| | NPS | Yellow | 490 | 234 |
| 5-Aminoquinoline | PS | Orange-red | 550 | 299 |
| | APS | Red | 630 | 230 |
| | NPS | Orange | 237 | 163 |
| 6-Aminoquinoline | PS | Brown oil | — | — |
| | APS | Yellow | 323 | 131 |
| | NPS | Yellow | 413 | 147 |
| 7-Aminoquinoline | PS | Red oil | — | — |
| | APS | Yellow | 790 | 445 |
| | NPS | Yellow | 94 | 32 |
| 8-Aminoquinoline | PS | Red oil | — | — |
| | APS | Light yellow | 930 | 530 |
| | NPS | Dark yellow | 368 | 323 |
| 4-Amino-2-methylquinoline | PS | Colourless | 860 | 325 |
| | APS | Colourless | 219 | 105 |
| | NPS | Colourless | 105 | 68 |

Table 4. *Precipitation of sulphate esters with 5-aminoacridine hydrochloride*

| Type of sulphate ester | Sulphate | Salt precipitation |
|---|---|---|
| Arylsulphates | 3-Methylphenyl sulphate | + |
| | Indoxyl sulphate | + |
| | 2-Amino-3-carboxyphenyl sulphate | + |
| | 2-Amino-5-carboxyphenyl sulphate | + |
| | 2-Amino-4-methylphenyl sulphate | + |
| | 2-Amino-4-chlorophenyl sulphate | + |
| | 3-Amino-3-nitrophenyl sulphate | + |
| | 4-Amino-3-phenylphenyl sulphate | + |
| | 6-Amino-3-methylphenyl sulphate | + |
| | 2-Amino-1:naphthyl sulphate | + |
| | 2′-Methyl-4-dimethylamino-*trans*-stilbene 3-sulphate | + |
| | 2′-Chloro-4-dimethylamino-*trans*-stilbene 3-sulphate | + |
| | 4-Dimethylaminoazobenzene 3-sulphate | + |
| Alkylsulphates | Dehydro*iso*androsterone sulphate | + |
| | *iso*Androsterone sulphate | − |
| | Ethyl sulphate | − |
| | Sinigrin* | − |
| Carbohydrate sulphates | Glucose 3-sulphate | − |
| | Glucose 6-sulphate | − |
| | Galactose 6-sulphate | + |
| | 1:2, 5:6-Di*iso*propylidene glucose 3-sulphate | + |
| | Laminarin sulphate | + |
| | Carragheenin* | + |
| | Fucoidin* | + |
| | Heparin* | + |
| | Chondroitin sulphate* | + |

* Biosynthetic specimens.

(ii) *Treatment with ion-exchange resin.* Amberlite IR-120 (H) was converted into the potassium form by treatment with 10% (w/v) KOH (300 ml./100 g. resin) for 30 min. After washing with water the resin was separated by filtration and stirred at 80° with a suspension of 5-aminoacridine *p*-nitrophenyl sulphate (2 g. in 400 ml. water). After 2 hr., the resin was separated and the filtrate concentrated *in vacuo* at 35° until about 10 ml. remained. On cooling, 1·2 g. (96% yield) of potassium *p*-nitrophenyl sulphate separated out. Repetition of the procedure with 5-aminoacridine *p*-acetylphenyl sulphate gave a 70% yield of the corresponding potassium salt.

Table 5. *Precipitation of certain urinary constituents with 5-aminoacridine hydrochloride*

| Urinary constituent | Salt precipitation |
|---|---|
| Uric acid | – |
| Urea | – |
| Glycine conjugates | |
| Hippuric acid | – |
| *p*-Hydroxyhippuric acid | – |
| *p*-Aminohippuric acid | – |
| Mercapturic acids | |
| L-2:5-Dichlorophenyl- | – |
| L-*p*-Iodophenyl- | – |
| L-*m*-Chloro- | – |
| L-Phenyl- | – |
| L-*p*-Bromophenyl- | – |
| L-*o*-Chlorophenyl- | – |
| L-2:4-Dichlorophenyl- | – |
| L-2:6-Dichlorophenyl- | – |
| Glucosiduronic acids | |
| *o*-Aminophenyl- | – |
| *m*-Aminophenyl- | – |
| *p*-Chlorophenyl- | – |
| Stilboestrol- | + |
| Dienoestrol- | + |
| Di*iso*propyl methyl- | – |
| 2-Ethyl hexanoyl- | – |
| Bornyl- | – |
| Benzoyl- | – |
| Other compounds | |
| Benzoic acid | – |
| Oxalic acid | – |
| Phenyl phosphate | + |
| *p*-Nitrophenyl phosphate | + |

### *Precipitation of other sulphate esters with 5-aminoacridine*

Table 4 lists other sulphate esters which were tested with a saturated solution of 5-aminoacridine hydrochloride at room temp. Compounds of known molecular weight were used at a concentration of 0·025 M. When molecular weights were unknown, 1% (w/v) solutions were used.

*Precipitation of possible urinary constituents with 5-aminoacridine.* One ml. of a solution (0·025 M) of the compound to be tested was mixed with 1 ml. of a saturated solution of 5-aminoacridine hydrochloride. The results are recorded in Table 5.

### *Recovery of arylsulphates from urine*

The addition of a saturated solution of 5-aminoacridine to normal rabbit and human urine gave precipitates approximating to 0·1 g./100 ml. and 0·06 g./100 ml., respectively. The precipitates contained 5-aminoacridine and appeared to consist in part of the mixed 5-aminoacridine salts of the normally occurring ethereal sulphates. Potassium *p*-chlorophenyl sulphate which had been added to rabbit urine (0·37 g./100 ml.) could be recovered almost quantitatively (92%) but was contaminated with the salts of other ethereal sulphates. However, when present in amounts more representative of detoxication experiments (0·15 g./100 ml. corresponding to a 12% sulphate conjugation of a total dose of 10 g. *p*-chlorophenol eliminated in 1·5 l. of urine; cf. Spencer & Williams, 1950) only about 10% of the added *p*-chlorophenyl sulphate could be recovered and this was heavily contaminated with other precipitated material. When added in the same concentration to a urine previously concentrated to 0·2 vol., only about 45% of the *p*-chlorophenyl sulphate could be recovered and this also was heavily contaminated. These low recoveries may possibly be due to the suppression of precipitation by inorganic ions, as noted by Egami (1939).

The most satisfactory method of precipitating arylsulphates from rabbit urine necessitated a preliminary treatment of the urine as detailed in the next section. In this way 90% recoveries of added arylsulphate could be made and the product was substantially pure after two recrystallizations.

### *The isolation of 5-aminoacridine p-chlorophenyl sulphate from rabbit urine*

In an experiment designed to test the practical application of 5-aminoacridine to the separation of urinary arylsulphates, an attempt was made to isolate *p*-chlorophenyl sulphate from the urine of rabbits receiving *p*-chlorophenol.

*p*-Chlorophenol (0·8 g.) suspended in water was fed to each of ten rabbits. The pooled 16 hr. urine (650 ml.) was filtered and concentrated *in vacuo* at 40°. The concentrated urine (20 ml.) was saturated with ammonium sulphate and extracted with acetone (1 l.). After filtering, the filtrate was concentrated to 200 ml. *in vacuo* and a further 1 l. of acetone was added. The precipitated glucuronide gum was discarded. The filtrate was concentrated *in vacuo* and the acetone treatment was repeated. After filtering, acetone was removed from the glucuronide-free solution *in vacuo*. An excess of a warm saturated solution of 5-aminoacridine hydrochloride was added with stirring and scratching when 5-aminoacridine *p*-chlorophenyl sulphate separated as a yellow crystalline mass. The crystals were washed with ice-cold water and recrystallized 4 times from water (charcoal). (Found: C, 55·8; H, 4·1; N, 6·6. $C_{19}H_{15}N_2O_4SCl$, $0{\cdot}5H_2O$ requires C, 55·4; H, 3·9; N, 6·8.) The structure of the compound was confirmed by hydrolysis with N-HCl followed by the isolation of the liberated *p*-chlorophenol as the corresponding *p*-toluenesulphonate (m.p. and mixed m.p. 70–71°).

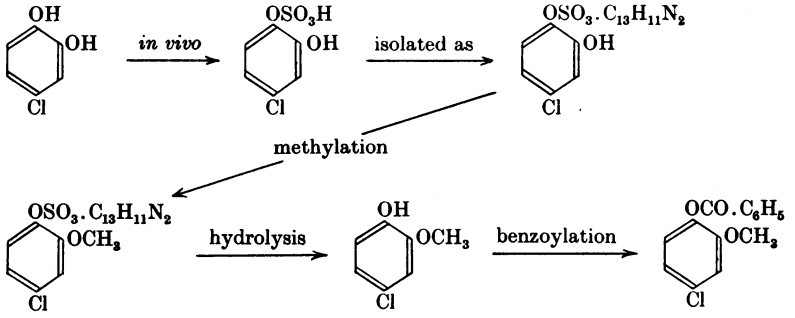
*The isolation and characterization of the 4-chlorocatechol monosulphate arising from the metabolism of chlorobenzene and 4-chlorocatechol*

In rabbits the glucuronic acid conjugation of dihydric phenols, when a third substituent group (other than –OH) is present, results in the conjugation of the –OH group which is furthest removed from the third substituent group (Dodgson, 1950; Dodgson & Williams, 1949; Dodgson, Smith & Williams, 1950). Attempts to extend these findings to the ethereal sulphate conjugation have been only partly successful. Dodgson & Williams (1949) obtained some evidence to show that the conjugation of sulphuric acid with 4-chlorocatechol also involved the –OH group furthest removed from the substituent Cl atom, but unequivocal proof was not obtained since 4-chlorocatechol monosulphate was not isolated in crystalline form. 4-Chlorocatechol monosulphate is also an important metabolite of chlorobenzene (Smith, Spencer & Williams, 1950), but its structure was not established with certainty (Spencer, 1950).

It has now been possible to ascertain the configuration of these biosynthetic catechol monosulphates following their isolation as crystalline 5-aminoacridine salts.

*The isolation of 5-aminoacridine 4-chlorocatechol monosulphate from the urine of rabbits receiving 4-chlorocatechol.* 4-Chlorocatechol (9·2 g.; prepared according to Willstätter & Müller, 1911) was fed to twelve rabbits over a period of 2 days. The urine (2 l.) was concentrated *in vacuo* at 35° and the glucuronides removed as described in the previous section. A saturated solution of basic lead acetate was added to the glucuronide-free concentrate until no further precipitation occurred and the precipitate, containing the lead salt of 4-chlorocatechol monosulphate (Spencer, 1950), was separated and washed well with water. The precipitate was suspended in water and decomposed with $H_2S$. The lead sulphide was separated and dissolved $H_2S$ removed from the filtrate by aeration. The solution was made alkaline to litmus with $K_2CO_3$, concentrated to 50 ml. *in vacuo* at 35° and treated with a slight excess of a warm saturated solution of 5-aminoacridine hydrochloride. The yellow precipitate (2·8 g.) of the 5-aminoacridine salt of 4-chlorocatechol was recrystallized thrice from 50% (v/v) ethanol (charcoal). (Found: C, 51·7; H, 4·1. $C_{19}H_{15}O_5N_2SCl, H_2O$ requires C, 52·2; H, 3·9.) The salt gave 4-chlorocatechol (identified as the di-*p*-toluenesulphonate, m.p. and mixed m.p. 114–116°) after hydrolysis with 0·5 N-HCl for 30 min. at 100°.

*The structure of 5-aminoacridine 4-chlorocatechol monosulphate.* The salt (0·41 g.) was suspended in 200 ml. of warm ethanol and treated for 24 hr. with two successive portions (300 ml.) of an ethereal solution of diazomethane. Concentration *in vacuo* at 35° left a brown-red gum. (Found: $OCH_3$, 7·5; the monomethyl derivative of 5-aminoacridine 4-chlorocatechol monosulphate requires $OCH_3$, 7·2.) The methylated gum was hydrolysed by heating for 30 min. in a boiling-water bath with 10 ml. 0·5 N-HCl and the liberated methylated phenol extracted with ether. The ethereal solution was dried over anhydrous $Na_2SO_4$ and concentrated. The resulting syrup was benzoylated, the solid benzoyl derivative recrystallized thrice from ethanol and identified as 4-chloro-2-methoxyphenyl benzoate by comparison with an authentic specimen of the compound prepared according to Jona & Pozzi (1911) (m.p. and mixed m.p. 77–78°). The reactions are summarized below:




This confirmed the suggestion of Dodgson & Williams (1949) that conjugation occurred on the hydroxyl group furthest removed from the substituent Cl atom.

*The isolation and structure of 5-aminoacridine 4-chlorocatechol monosulphate from the urine of rabbits receiving chlorobenzene.* The 4-chlorocatechol monosulphate arising from chlorobenzene was isolated as the 5-aminoacridine salt using the experimental procedure described in the preceding section. During this procedure 4-chlorocatechol monosulphate was separated from the sulphates of *o*- and *p*-chlorophenol (minor metabolites of chlorobenzene; Azouz, Parke & Williams, 1953) by the lead acetate treatment, since *p*- and *o*-chlorophenyl sulphates do not form insoluble lead salts. The structure of 4-chlorocatechol monosulphate was established as 4-chloro-2-hydroxyphenyl sulphate by the methylation procedure previously described.

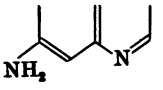
*The structure of synthetic 4-chlorocatechol monosulphate.* 4-Chlorocatechol monosulphate, prepared from 4-chlorocatechol by the method of Burkhardt & Lapworth (1926) but using sufficient chlorosulphonic acid to sulphate one hydroxyl group only, was shown by the same methylation

procedure to be identical with the 4-chlorocatechol monosulphate isolated after feeding chlorobenzene and 4-chlorocatechol.

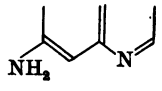
## DISCUSSION

The formation of relatively insoluble 5-aminoacridine salts provides a convenient method of isolating urinary biosynthetic arylsulphates. Despite preliminary treatment of the urine, contamination with the salts of the arylsulphates which are normally present in urine occurs. When an arylsulphate is present in urine in high concentration, e.g. as a result of feeding *p*-chlorophenol, the corresponding aminoacridine salt is fairly easily purified by recrystallization. However, when the arylsulphate required is present in small amounts only, the degree of contamination is considerably increased and purification is difficult. When two or more arylsulphates are present in appreciable amounts, such as *p*-chlorophenyl and 4-chlorocatechol sulphates after feeding chlorobenzene, preliminary separation is essential before precipitation with the base. Although 5-aminoacridine has been used in the present work other *N*-heterocyclic bases may prove of greater value in specific cases.

On the basis of the precipitating action of 5-, 6-, 7- and 8-aminoquinolines with certain sulphate esters, Egami (1940) suggested that the presence in the base of the group



was necessary for the formation of insoluble salts. Trypaflavin (euflavin) and all the other aminoacridines tested by Egami possessed this group. Egami also noted a parallelism between the ability of sulphate esters to give precipitates with bases possessing the essential group and their susceptibility to hydrolysis by aryl-, glyco- or chondro-sulphatase. On these grounds he suggested that the



group may be present in the various sulphatase enzymes and that it was the group responsible for the binding of enzyme and substrate.

Further results, presented here, on the precpitation reaction between sulphate esters and *N*-heterocyclic bases are not in accord with Egami's conclusions. For instance, 5-aminoacridine, which does not possess Egami's essential group, gave salts with a large variety of sulphate esters, which had solubilities similar to the corresponding euflavin salts. Whereas Egami (1940) found that only 7-aminoquinoline gave a precipitate with PS, the present work showed that all the aminoquinolines, including the 2-, 3- and 4-isomers, which were not tested by Egami, gave insoluble salts with PS, APS and NPS. These results were obtained with a concentration of base and sulphate approximately 16 times greater than that used by Egami (1940). It seems probable that the varying abilities of different bases to precipitate sulphate esters does not depend absolutely on the presence of a specific grouping, but depends on the solubilities of the salts formed and the initial concentrations of the reactants. Egami's (1940) conclusions thus seem to result from a fortuitous selection of experimental conditions. Thus his inability to precipitate sulphate esters with acridine was probably due to the extremely low solubility of acridine in water, so that the solubility product of the salt was not exceeded. A suitably concentrated solution of acridine, as the hydrochloride, gave relatively insoluble salts with PS, APS and NPS.

The parallelism noted by Egami (1940) between the precipitation of sulphate esters and their susceptibility to hydrolysis by sulphatases also seems to depend on the conditions of precipitation chosen. Thus, at the concentration used, 5-aminoacridine does not give precipitates with glucose 3- and 6-sulphates, both of which are hydrolysed by glycosulphatase (Dodgson & Spencer, 1954), whereas euflavin precipitates both compounds. Dehydro*iso*androsterone sulphate is hydrolysed by a sulphatase present in limpets (Stitch & Halkerston, 1953) but is not precipitated by euflavin or safranine unless very high concentrations of base and sulphate are used. Ethyl sulphate, which is apparently not attacked by any sulphatase, can be precipitated by suitable concentrations of 5-aminoacridine, euflavin or safranine.

However, sulphate esters do appear to be preferentially precipitated by *N*-heterocyclic bases since only two glucosiduronates and the arylphosphates out of a number of organic acids tested (Table 5) gave relatively insoluble salts. It therefore seems likely that, as Egami (1940) suggests, sulphate esters may be bound to sulphatases through basic groups present in the enzyme. After studying the effects of group-specific protein reagents on the 'soluble' sulphatase (cf. Dodgson, Spencer & Thomas, 1955) of rabbit liver, Maengwyn-Davies & Friedenwald (1954) suggested that basic groups (probably imidazole) may be associated with enzyme activity.

## SUMMARY

1. Safranine, euflavin and 5-aminoacridine gave crystalline salts of low solubility with phenyl, *p*-acetylphenyl and *p*-nitrophenyl sulphates. The

aminoquinoline salts of the same sulphates were more soluble.

2. 5-Aminoacridine gave relatively insoluble salts with many sulphate esters but, with two exceptions, did not precipitate a number of glucosiduronic, mercapturic and hippuric acids at the concentrations used.

3. 5-Aminoacridine was used to isolate the arylsulphates formed after feeding *p*-chlorophenol, chlorobenzene and 4-chlorocatechol to rabbits.

4. The 4-chlorocatechol monosulphates isolated from the urines of rabbits receiving chlorobenzene or 4-chlorocatechol were shown to be 4-chloro-2-hydroxyphenyl sulphate. The synthetic monosulphate had the same structure.

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