1335. *The Reaction of Mepacrine with Thiols*

By F. Wild and J. M. Young

The reaction between mepacrine and thiols at room temperature, which yields the corresponding 9-thioacridine (IV), has been studied. Cysteine reacted with mepacrine at physiological temperature and pH to give the bisacridinylcysteine (IX). The stability of this product has been compared with that of certain *N*-acridinylamino-acids (XI). The reaction between mepacrine and glutathione has also been investigated.

Mepacrine, 6-chloro-9-(4-diethylamino-1-methylbutylamino)-2-methoxyacridine dihydrochloride (I), originally introduced [1] and extensively used as an antimalarial, is also active against a variety of other diseases. Thus, apart from having a marked therapeutic effect against equine encephalomyelitis in the mouse,[2] it has found clinical use in the treatment of *Lupus erythematosus*,[3] *Taenia saginata* infestations,[4] refractory *petit mal*,[5] and recurrent neoplastic effusions.[6] Of particular interest is the slight, but definite, anti-tumour activity, which is reviewed elsewhere.[7] The mode or modes by which mepacrine exerts these actions remains unknown. Few metabolites have been identified, but of those that have, two have lost the diamine side-chain.[8] The removal of the side-chain *in vitro* by acid or alkaline

[1] H. Mauss and F. Mietzsch, *Klin. Wochenschr.*, 1933, **12**, 1276.
[2] E. W. Hurst, P. Melvin, and J. M. Peters, *Brit. J. Pharmacol.*, 1952, **7**, 455.
[3] E. L. Dubois, *A.M.A. Arch. Dermat.*, 1955, **71**, 570.
[4] L. S. Goodman and A. Gilman, "The Pharmacological Basis of Therapeutics," Macmillan, New York, 2nd edn., 1955, p. 1155.
[5] W. A. Sibley, *New England J. Med.*, 1962, **267**, 332.
[6] A. Gellhorn, J. Zaidenweber, J. Ultmann, and E. Hirschberg, *Dis. Chest*, 1961, **39**, 165.
[7] J. M. Young, F. Wild, and I. Simon-Reuss, *Brit. J. Cancer*, 1965, **19**, 370.
[8] D. Ll. Hammick and S. F. Mason, *Nature*, 1945, **156**, 718.

hydrolysis requires lengthy heating under reflux.[9] Asquith, Hammick, and Williams observed, however, that in mildly basic solution at room temperature, mepacrine reacted very rapidly with hydrogen sulphide, the side-chain being split off.[10] The strikingly mild conditions under which the last reaction occurs suggest that physiological temperature and pH might also be suitable. Further, could a reaction with thiol compounds in general be demonstrated, then this might not only provide a basis for the wide spectrum of activity, but would also be a common factor with the nitrogen mustards, ethyleneimines, and alkyl sulphonates currently used in the treatment of cancer, and which have been postulated to exert their effect by reacting with vital cellular thiols.[11] In the case of Myleran, the major urinary metabolite is derived from a reaction with the thiol group of cysteine or cysteine-containing peptides.[12] A similar reactivity has also been observed with 4-(4-diethylaminostyryl)quinoline, which causes some regression of lymphoma 8 in rats.[13] We report here the results of a study of the reaction *in vitro*, under mild conditions, between mepacrine and thiols.

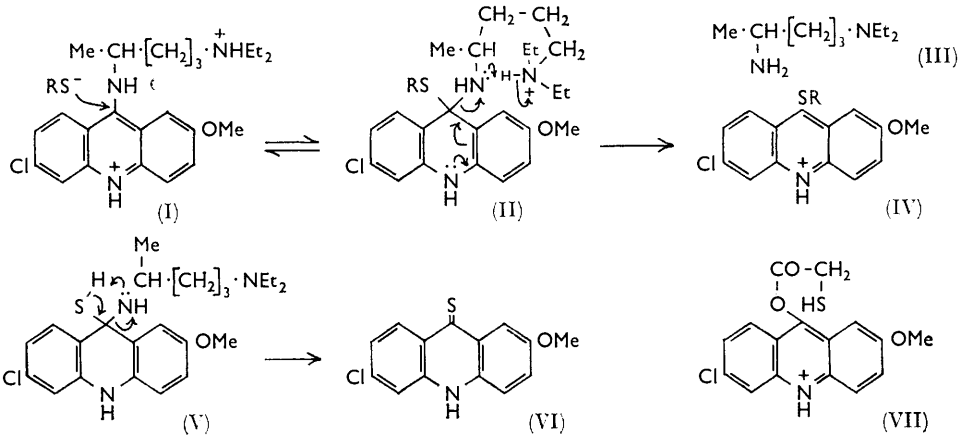
Asquith, Hammick, and Williams reported that the reaction between 9-aminoacridines and hydrogen sulphide occurred under optimum conditions in mildly basic solution.[10] These authors generally used ammoniacal ethanol. We have found, however, that this medium had a disadvantage where, as in the case of ethanethiol and thiophenol, the reaction mixture had to be left for several days at room temperature, since appreciable amounts of 9-amino-6-chloro-2-methoxyacridine (X; R = H) were formed. Thus pyridine, in which mepacrine was degraded as rapidly by hydrogen sulphide as in ammoniacal ethanol, was generally used as base catalyst. In pyridine or ethanolic pyridine, mepacrine reacted with ethanethiol at room temperature to give 6-chloro-9-ethylthio-2-methoxyacridine (IV; R = Et). However, in contrast to the reaction with hydrogen sulphide, the rate of reaction was greatly enhanced when the mepacrine was used in the form of the dihydrochloride rather than as the free base. Thus, after 7 days, the reaction between mepacrine free base and ethanethiol yielded only 2% of the ethylthioacridine (IV; R = Et), whereas a 93% yield of the same compound was obtained from that with the dihydrochloride. In the case of mepacrine free base, only a slight improvement of the yield was noted when 4% pyridine in ethanol was used in place of pyridine alone. The satisfactory yield, *ca.* 50%, obtained from a solution, necessarily dilute, of the dihydrochloride in pyridine, demonstrated that ethanol was not essential for the success of the reaction, but the much greater solubility of the dihydrochloride in ethanolic pyridine makes this the medium of choice. The reaction between the dihydrochloride and ethanethiol also proceeded, although at a slower rate, when pyridine was absent. This was to be expected, since the products (III and IV), once formed, have a free basic centre, which will catalyse further reaction. Thus a solution of the dihydrochloride and ethanethiol in ethanol yielded, after 7 days at room temperature, 50% of the ethylthioacridine (IV; R = Et), as compared with 93% from an equivalent mixture with pyridine present.

Similar results to those above were obtained when the ethanethiol was replaced by thiophenol, the product in this case being the phenylthioacridine (IV; R = Ph). This product was formed more slowly than the ethylthioacridine (IV; R = Et), and solutions of mepacrine free base and thiophenol in pyridine or ethanolic pyridine, after several weeks at room temperature, showed, on chromatography, only traces of it. The yields of the phenylthioacridine (IV; R = Ph) from reactions with the dihydrochloride in ethanolic pyridine were variable, and apparently depended on the rate at which the thiophenol was converted into the disulphide, thus preventing further reaction with the mepacrine. However, yields of up to 70% were obtained after 40 days. Here again the ethanol was

[9] O. J. Magidson and A. M. Grigorowski, *Ber.*, 1936, **69**, 396.
[10] R. S. Asquith, D. Ll. Hammick, and P. L. Williams, *J.*, 1948, 1181.
[11] G. Calcutt and T. A. Connors, *Biochem. Pharmacol.*, 1963, **12**, 839.
[12] J. J. Roberts and G. P. Warwick, *Nature*, 1959, **183**, 1509.
[13] C. T. Bahner, C. Cook, W. Longmire, and S. V. Hagen, *J. Org. Chem.*, 1961, **26**, 2134.

not essential for the reaction, and chromatography showed that comparable yields could be obtained from solutions of the dihydrochloride and thiophenol in pyridine alone.

The very much greater reactivity of the dihydrochloride of mepacrine as compared to the free base is striking. The increased rate of nucleophilic substitution which may result from the protonation of heterocyclic compounds was first recognised by Banks,[14] but it had been noted some years earlier that in the reaction between 9-alkoxyacridines and amines, highest yields were obtained when one of the reactants was protonated.[15,16] The catalytic effect of the hydrogen chloride was subsequently demonstrated.[17] The first



stage in the reaction with thiols is thus attack by the thiolate anion, whose formation is promoted by the pyridine present, at the 9-position in (I), leading to the intermediate (II). The formation of compound (II) is necessary before the 9-amino group, which is then no longer in the plane of the acridine ring, can accept the proton which allows it to separate easily as the primary amine (III). In mepacrine dihydrochloride (I) itself, both the ring nitrogen and the 9-amino group are involved in a strongly resonance-stabilised cation,[18] the addition of a second proton to the system occuring only in 17M-sulphuric acid.[19] Two possibilities are open for the gain of the proton by the 9-amino group of compound (II). In protic solvents it would presumably come primarily from the solvent, but a second possibility is depicted in (II) ⟶ (III), where the side-chain, no longer constrained to be extended away from the resonant cation involving the 9-amino group,[20] may undergo folding which allows the transfer of a proton between the two basic centres. Inspection of a model of compound (II) shows that this may occur under optimum steric conditions. The success of the reaction of mepacrine dihydrochloride with ethanethiol and thiophenol in pyridine alone, suggests that this second possibility may play a significant role. This particular advantage of the mepacrine side-chain will also be present in homologues $CH_3{\cdot}CH(NH{-}){\cdot}(CH_2)_n{\cdot}NEt_2$ where $n \geqslant 2$. However, when $n > 5$, the folding required would be expected to lead to a decrease in the rate of proton transfer, thus favouring (II) ⟶ (I) as compared to (II) ⟶ (III) + (IV). It is therefore an interesting parallel that acridines showing antimalarial activity (a), generally have a diamine side-chain and (b), with side-chains of the type above, the optimum activity occurs[21] when $n = 3$ or 4.

[14] C. K. Banks, *J. Amer. Chem. Soc.*, 1944, **66**, 1127.
[15] N. S. Drosdow and O. M. Cherntzow, *J. Gen. Chem.* (*U.S.S.R.*), 1935, **5**, 1736.
[16] D. J. Dupré and F. A. Robinson, *J.*, 1945, 549.
[17] H. J. Barber, J. H. Wilkinson, and W. G. H. Edwards, *J. Soc. Chem. Ind.*, 1947, **66**, 411.
[18] S. J. Angyal and C. L. Angyal, *J.*, 1952, 1461.
[19] J. L. Irvin, R. W. McQuaid, and E. M. Irvin, *J. Amer. Chem. Soc.*, 1950, **72**, 2750.
[20] J. L. Irvin and E. M. Irvin, *J. Amer. Chem. Soc.*, 1950, **72**, 2743.
[21] K. C. Blanchard and L. H. Schmidt, in " A Survey of Antimalarial Drugs," ed. F. Y. Wiselogle, J. W. Edwards, Ann Arbor, Michigan, 1946.

The importance of having a proton available to allow the separation of the diamine side-chain (compound III) before the reverse reaction, (II) ⟶ (I), occurs, is demonstrated by the very rapid reaction of mepacrine, free base or dihydrochloride, with hydrogen sulphide in pyridine at room temperature, the thione (VI) starting to separate after only a few minutes. Here in the intermediate (V) the thiol proton can be transferred directly to the adjacent amino-group.

The use of ethanolic pyridine as the reaction medium was not so successful when the series of reactions with mepacrine dihydrochloride was extended to thioglycollic acid. After two days at room temperature a number of products were present in solution, although the 9-carboxymethylthio-6-chloro-2-methoxyacridine (IV; R = $CH_2 \cdot CO_2H$) commenced to separate in a fairly pure state. Its identity was confirmed by comparison with a sample obtained from the reaction between the thione (VI) and chloroacetic acid. The m. p. of the analytically pure crystalline sample was lower, however, than that of amorphous preparations showing an identical infrared (i.r.) spectrum. The same effect was also observed later with the *N*-acridinylmethionine (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$). An alternative synthesis of the carboxymethylthioacridine (IV; R = $CH_2 \cdot CO_2H$) from 6,9-dichloro-2-methoxy-acridine and thioglycollic acid in phenol, afforded a product identical with those above. The formation of numerous by-products in the reaction of mepacrine dihydrochloride with thioglycollic acid was not affected by exchanging the pyridine for other organic bases. The only secondary product identified was the thione (VI), which was present in 1 and 3 month-old reaction mixtures in 8 and 25% yield respectively. In the latter mixture, the initial precipitate had partly dissolved, and the i.r. spectrum of the solid material remaining showed no absorption at 1712 $cm.^{-1}$ This suggests that some of the substances detected are decomposition products of the initially formed carboxymethylthioacridine (IV; R = $CH_2 \cdot CO_2H$). It would indeed be difficult to account for the presence of the thione (VI) in any other way.

As an alternative to the basic conditions, the reaction with thioglycollic acid was investigated using mepacrine free base in glacial acetic acid. The interesting properties of acetic acid as a solvent for nucleophilic substitution reactions have recently been noted by Reinheimer, Gerig, Garst, and Schrier.[22] In the case of thioglycollic acid, this solvent was much better than ethanolic pyridine, an 80% yield of the carboxymethylthioacridine (IV; R = $CH_2 \cdot CO_2H$) being obtained after only 4 days at room temperature. However, the reaction between mepacrine free base and ethanethiol in acetic acid was very slow, only 2% of the ethylthioacridine (IV; R = Et) having been formed after a week. Hydrogen sulphide, passed through solutions of mepacrine, free base or dihydrochloride, in acetic acid at room temperature, produced detectable amounts of the thione (VI) only after some 6 hr. However, at 80° a reasonable yield was obtained in 3—4 hr. This observation contrasts with the report that mepacrine does not react with hydrogen sulphide under acid conditions.[10] The reaction between mepacrine free base and thiophenol in acetic acid–ether was surprisingly successful, a 28% yield of the phenylthioacridine (IV; R = Ph) having formed after 20 days at room temperature. The product was isolated without difficulty, whereas samples obtained from the reaction with mepacrine dihydrochloride in ethanolic pyridine were frequently troublesome to purify.

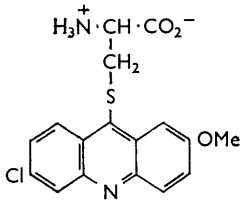
The mechanism of the reactions of the free base of mepacrine in acetic acid is probably different from that of the dihydrochloride in basic media. It seems likely that the 9-acetate is an intermediate, since it is then possible to explain the great reactivity of thioglycollic acid as compared to that of the other thiols examined. The key intermediate is probably of the form (VII), which might arise either by direct replacement of the diamine side-chain by the thioglycollate residue or by exchange of the thioglycollate with the 9-acetate. The thiol group is thus brought into a position favourable for rearrangement, forming the carboxymethylthioacridine (IV; R = $CH_2 \cdot CO_2H$).

The mild conditions under which the reaction between mepacrine and thiols proceeds

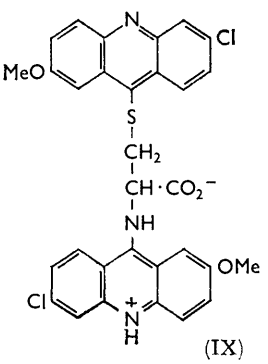
[22] J. D. Reinheimer, J. T. Gerig, R. Garst, and B. Schrier, *J. Amer. Chem. Soc.*, 1962, **84**, 2770.

must be emphasised, since under forcing conditions the reverse reaction can be made significant. This point is illustrated by the results of Kitani,[23] who studied the reaction of the diamine (III) with various 9-amino and 9-thioacridines. Thus, even the very stable 9-amino-6-chloro-2-methoxyacridine (X; R = H) gave a 46% yield of mepacrine after heating with the diamine (III) at 160—170° in ethanolic acetic acid for 6 hr. Mepacrine could also be obtained, in 29% yield, by heating the ethylthioacridine (IV; R = Et) with the diamine (III) under reflux in n-butanol–lead tetra-acetate for 30 hr.

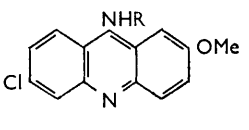
The conclusion that reaction between mepacrine and thiols will be favoured under conditions where both the base is protonated and the thiol ionised, was further confirmed by the smooth reaction between mepacrine and cysteine in aqueous buffer, pH 7·1, at 37°. Benesch and Benesch [24] have calculated that at pH 7·4 and 23°, cysteine is 6% in the form of the thiolate anion. At the same pH the resonant ring-N/9-amino-group system of



(VIII)



(IX)



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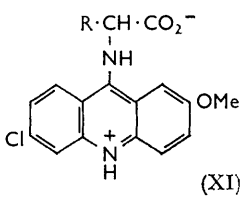


(XI)

mepacrine, pK_a 7·69, and the side-chain terminal diethylamino-group, pK_a 10·18, will be 67 and 99%, respectively, in the protonated form. The product from the reaction was not, however, the *S*-acridinylcysteine (VIII), to be expected by analogy with the course of the reactions with thiols reported above, but was established to be *NS-bis* (6-chloro-2-methoxy-9-acridinyl)-L-cysteine (IX). This product was decomposed by heat, and by boiling with acids and bases. Heated under reflux in acetic acid, two major products were formed, the thione (VI) and the acetate of an acridine base $C_{16}H_{13}ClN_2O_2$, which on pyrolysis yielded small amounts of 9-amino-6-chloro-2-methoxyacridine (X; R = H). On the basis of this degradation and the i.r. spectrum, the base was identified as the acetylaminoacridine (X; R = CO·Me). This structure was confirmed by the synthesis of compound (X; R = CO·Me) by acetylation of the amino-acridine (X; R = H) using the calculated amount of acetic anhydride in pyridine.[25] Acetylation of the 9-amino group to give compound (X; R = CO·Me), rather than at the ring nitrogen in the tautomeric imino form, has been demonstrated in the case of 9-aminoacridine.[26] The degradative evidence supports the presence of both a 9-acridinyl-nitrogen and a 9-acridinyl-sulphur bond in the mepacrine–cysteine reaction product. The solubility in ethanol–dilute alkali, virtually the only solvent, is consistent with the presence of the carboxylic acid group.

Confirmation of the structure (IX) was obtained from degradative studies with hydrogen sulphide. Previously only the degradation of 9-aminoacridine derivatives has been investigated,[10] but our experinece is that 9-thioacridines are also decomposed, yielding the thiol and the corresponding acridan-9-thione. Treatment of a suspension of the product (IX) in aqueous ethanolic sodium carbonate with hydrogen sulphide yielded the thione

[23] K. Kitani, *J. Chem. Soc. Japan*, 1954, **75**, 477.
[24] R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 1955, **77**, 5877.
[25] J. M. Wilkinson and I. L. Finar, *J.*, 1946, 115.
[26] A. Albert and R. Goldacre, *J.*, 1943, 454.

(VI) and cysteine, which was identified chromatographically. Compound (IX) was negative to both the iodine–azide reagent [27] (for the thiol group) and to ninhydrin. In experiments on a larger scale, other weak ninhydrin-positive spots were observed. The zwitterion structure of compound (IX) was inferred from the absence of un-ionised carboxyl absorption in the i.r. spectrum, but some samples showed a weak band near 1710 cm.$^{-1}$ The presence of both ionised and non-ionised carboxyl absorption in the i.r. spectrum of non-crystalline amino-acids has been reported previously.[28]

The bisacridinylcysteine (IX) separated from the mepacrine–cysteine reaction mixture in a nearly pure state, offering no clue to the intermediates involved in its formation. A two-step mechanism involving reaction with the thiol group of cysteine to form the *S*-acridinylcysteine (VIII), followed by attack of the amino-group of (VIII) on a second molecule of mepacrine, would imply at least a moderate lifetime for the intermediate (VIII). However, chromatography of the filtrate from the reaction mixture gave no ninhydrin- or iodine–azide-positive spots coinciding with fluorescence or ultraviolet absorption. In any case, direct nucleophilic attack on mepacrine by an amino-acid amine group, which at this pH will be almost entirely in the protonated form, would be expected to be very slow. This was verified by the rate of reaction of mepacrine with glycine, DL-α-alanine, and methionine in phosphate buffer, pH 7·1, at 37°. The yields, after 10 days, of the *N*-acridinylglycine (XI; R = H), the *N*-acridinyl-α-alanine (XI; R = Me), and the *N*-acridinylmethionine (XI; R = CH_2·CH_2·S·CH_3) were 10, 1—1·5, and 2% respectively. Samples of the acridinyl-α-alanine (XI; R = Me) and the acridinylmethionine (XI; R = CH_2·CH_2·S·CH_3) were also prepared by the reaction of 6-chloro-2-methoxy-9-phenoxy-acridine with the amino-acid in phenol. This reaction does not obey the rule [15,16] that in the preparation of 9-aminoacridine derivatives much better yields are obtained when one of the reactants is protonated, thus providing acid catalysis. Here the product (XI) of the reaction is a zwitterion, and a weaker base than the amino-acid from which it was derived, as demonstrated by the observation that *N*-(9-acridinyl) glycine hydrochloride is unstable in aqueous solution.[16] Thus any hydrogen chloride present will, as the product forms, largely go to protonating the amino-acid used as starting material, and thus eventually lead to a slowing of the reaction by reducing the proportion of the amino-acid in the form with the amino-group uncharged. In fact the acridinyl-α-alanine (XI; R = Me) was isolated from the reaction between 6-chloro-2-methoxy-9 phenoxyacridine and the amino-acid in 57% yield, and in 51% yield from the amino-acid and 6,9-dichloro-2-methoxyacridine (the 6-chloro-2-methoxy-9-phenoxyacridine hydrochloride being formed intermediately [9]). In the synthesis of the acridinylmethionine (XI; R = CH_2·CH_2·S·CH_3) the former method led to a very considerable improvement in the yield, 89% as compared to 35—40%. Here the presence of the hydrogen chloride may lead to some decomposition of the product, since lengthening the reaction time beyond a certain point caused a decrease in yield.

The weak basic properties of the acridinyl-α-alanine (XI; R = Me) were used in the preparation of a pure sample. The acetate, which separated on recrystallisation from acetic acid, decomposed readily on heating at reduced pressure, regenerating the zwitterion. Pure crystalline samples of the acridinylmethionine (XI; R= CH_2·CH_2·S·CH_3) had m. p. 182°. However, many amorphous preparations had m. p. up to 192°, but recrystallisation brought this down in stages to a constant 182°, Conversely, reprecipitation of a crystalline specimen gave an amorphous sample m. p. 187—188°.

The stability of the acridinylamino-acids (XI) towards recrystallisation from acetic acid contrasts with the ease with which this reagent decomposed the bisacridinylcysteine (IX). Heating the acridinylmethionine (XI; R = CH_2·CH_2·S·CH_3) under reflux in glacial acetic acid for 4 hr. gave only traces of decomposition products. Differences of

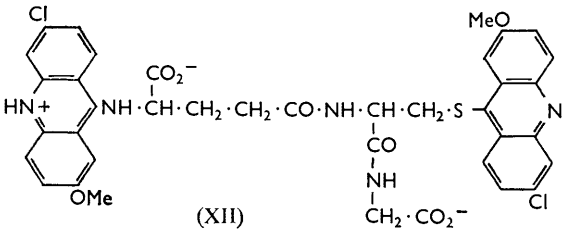
[27] F. Feigl, " Spot Tests in Organic Analysis," Elsevier, Amsterdam, 6th edn., 1960, p. 242.
[28] L. J. Bellamy, " The Infrared Spectra of Complex Molecules," Methuen, London, 1954, p. 207.

behaviour were also observed when these compounds were heated under reflux in dimethylformamide–triethylamine. The bisacridinylcysteine (IX) was rapidly decomposed, the major, and only identified, product being the thione (VI). The acridinylamino-acids (XI) were also decomposed, although more slowly. The product from the acridinylglycine (XI; R = H) was 6-chloro-2-methoxy-9-methylaminoacridine (X; R = Me). Similarly the acridinyl-α-alanine (XI; R = Me) yielded the decarboxylated derivative (X; R = Et). The acridinylmethionine (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$) was also decomposed, but the oil resulting was not identified.

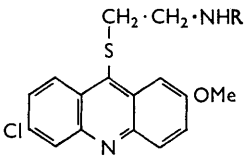
The methylaminoacridine (X; R = Me) initially isolated from the reaction mixture recrystallised to constant m. p. 135—137°, but sublimation raised this to 175° (lit.,[23] 174°). Attempts to repeat the preparation of the pure lower m. p. isomer were unsuccessful, and the original sample after standing in air for several weeks and being redried had m. p. 172—173°. The notable difference between the i.r. spectra (Nujol) of the two isomers is the presence of a distinct water band in that of the lower m. p. form, together with a shift of the –NH stretch band to shorter wavelength. This suggests that its formation is dependent on a hydration effect.

As was to be expected from the success of the mepacrine–cysteine reaction, mepacrine also reacted with glutathione (GSH) in buffer, pH 7·1, at 37°. Here again the product, in some preparations crystalline, was negative to both the ninhydrin and iodine–azide reagents. Evidence that reaction had occurred with mepacrine at the 9-position was afforded by the presence of the side-chain diamine (III) in the filtrate. The i.r. spectrum of the product was similar to that of the bisacridinylcysteine (IX). The strong bands in the spectrum of mepacrine dihydrochloride also occur, however, on or near strong absorption in the spectra of the product and compound (IX). The u.v. spectra of the bisacridinylcysteine (IX) and the above substance showed a very similar pattern, except in the 390—440 mμ region. Repeated washing of the product with water led to the steady extraction of mepacrine, together with some alkali-soluble material. Attempts to purify the substance by reprecipitation or recrystallisation were unsuccessful. Treatment of a suspension of the substance in ethanolic pyridine with hydrogen sulphide yielded the thione (VI) and two ninhydrin-positive products, which were identified by chromatography in three solvent systems as oxidised glutathione (GSSG) and the side-chain diamine (III).

On the basis of the above evidence the product has been tentatively identified as a complex of unknown proportions between *NS*-bis(6-chloro-2-methoxy-9-acridinyl)glutathione (XII) and mepacrine. The formation of GSSG rather than GSH on hydrogen sul-



(XII)



(XIII)

phide degradation is surprising; GSH treated in the same way gave only a little GSSG. On the other hand, the degradation of a suspension of the mepacrine–GSH reaction product in aqueous sodium carbonate yielded, after electrolytic desalting, predominately GSH and the diamine (III), and only a little GSSG. Other ninhydrin-positive spots were present. Further evidence for the presence of mepacrine as a complex with the product was afforded by the hydrogen sulphide degradation of a sample which had been three times reprecipitated from alcoholic alkali with acid. Here the spot due to the diamine (III) was absent, although the amounts of two unidentified ninhydrin-positive substances were substantially increased.

The complexing of the mepacrine–GSH reaction product by mepacrine apparently leads to its greater solublity as the concentration of mepacrine increases. Thus solid separated

from an equimolar mepacrine–GSH solution at pH 7·1 within 15 hr., but an equivalent mixture containing an extra mole of mepacrine showed no precipitate until after 3 days. A similar effect has been observed by Štastný and Hořejší where albumin, which was complexed and precipitated from blood plasma by 9-aminoacridine or trypaflavine, remained in solution at higher dye concentrations.[29]

The only ninhydrin-positive compounds present in the filtrate from the mepacrine–GSH reaction mixture, after electrolytic desalting, were GSH, GSSG, and the diamine (III). GSH and GSSG were the only iodine–azide positive spots. Thus, as in the reaction with cysteine, there was no evidence for any intermediates involved in the formation of the product. However, the rate of the reaction of mepacrine with cysteine is similar to that of mepacrine dihydrochloride with thioglycollic acid in ethanolic pyridine, which suggests that the initial and rate-determining step is attack by the thiol group, leading to the *S*-acridinylcysteine (VIII). This configuration apparently represents an unstable intermediate, which rearranges before reacting with a second molecule of mepacrine. A similar type of rearrangement in the quinoline series has recently been reported by Peck,[30] where hydrolysis of *N*-[*N'*-(7-chloro-4-quinolyl)-*N'*-methylaminoethyl]phthalimide yielded not the primary amine, but the rearranged product 7-chloro-4-(2-methylaminoethyl)-aminoquinoline. To determine whether *S*-acridinyl derivatives of 2-mercaptoethylamine may be prepared, or whether *NS*-bisacridinyl derivatives result, we attempted the synthesis of 9-(2-aminoethylthio)-6-chloro-2-methoxyacridine (XIII; R = H). The first approach was made *via* the toluene-*p*-sulphonyl derivative (XIII; R = $SO_2 \cdot C_6H_4 \cdot CH_3$), prepared by condensing *N*-toluene-*p*-sulphonyl-2-chloroethylamine with the thione (VI) in the presence of sodium ethoxide. Confirmation that *S*-alkylation, rather than *N*-alkylation, occurs was afforded by the close similarity of the u.v. spectrum of the product with that of the ethylthioacridine (IV; R = Et) rather than with that of the thione (VI). However, attempts to obtain the free amine, or to isolate rearranged products after removal of the toluene-*p*-sulphonyl group, were unsuccessful. Much more promising results were obtained from preliminary experiments with the benzyloxycarbonyl derivative (XIII; R = $CO \cdot O \cdot CH_2 \cdot C_6H_5$), synthesised from the thione (VI) and *N*-benzyloxycarbonyl-2-bromoethylamine. Here removal of the benzyloxycarbonyl group by hydrogen bromide in acetic acid gave two major products. The first is degraded by hydrogen sulphide to give a ninhydrin-positive product, which shows it to be a derivative of 9-aminoacridine. The second has an i.r. spectrum identical with that of the product, at present uncharacterised, of the reaction of mepacrine with 2-mercaptoethylamine, and which on hydrogen sulphide degradation yields 2-mercaptoethylamine in about 50% yield. These observations thus lend support for structure (VIII) as the key intermediate in the reaction between mepacrine and cysteine.

If *S*-acridinyl derivatives of 2-mercaptoethylamine are in general unstable, then the number of products obtained on the hydrogen sulphide degradation of *NS*-bisacridinyl derivatives, *e.g.*, (IX), will not be a good test of purity, since cleavage of the acridine–nitrogen bond will leave an *S*-acridinyl-2-mercaptoethylamine, which may rearrange or decompose before attack on the acridine–sulphur linkage takes place. In general, however, the remarkably mild conditions under which hydrogen sulphide degrades aminoacridine derivatives,[10] whose synthesis from the primary amine offers no difficulty,[9,16] suggests that the 9-acridinyl group might find wide application for the protection of primary amines. The stability of amino-acridines in acid conditions and against alkali at room temperature, under which conditions other protecting groups can be removed,[31] underlines the potential utility where differential protection is required.

[29] M. Štastný and J. Hořejší, *Clinica Chim. Acta*, 1961, **6**, 782.
[30] R. M. Peck, *J. Org. Chem.*, 1962, **27**, 2677.
[31] L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, 1962, **84**, 3887; L. Zervas, I. Photaki, and N. Ghelis, *J. Amer. Chem. Soc.*, 1963, **85**, 1337; F. M. Callahan, G. W. Anderson, R. Paul, and J. E. Zimmerman, *J. Amer. Chem. Soc.*, 1963, **85**, 201; D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1952, **17**, 1564.

Experimental

Mepacrine dihydrochloride was obtained from I.C.I. Ltd., and used without further purification. I.r. spectra were obtained for Nujol mulls. Light petroleum refers to the fraction of b. p. 80—100°. Chromatography was carried out on Whatman No. 1 paper, unless otherwise stated, in the following solvent systems: (A), n-butanol–acetic acid–water, 4 : 1 : 5; (B), phenol saturated with water–ethanol–conc. ammonia, 15 : 14 : 1; (C), phenol saturated with water, in the presence of 50% aqueous acetic acid; and (D), pyridine–acetic acid–water, 10 : 7 : 3. The ninhydrin reagent was an 0·2% solution in n-butanol–2N-acetic acid, 19 : 1. The iodine–azide reagent was prepared according to the method of Smith.[32] The strength of the spots is denoted by, (vw) very weak, (w) weak, and (s) strong.

Reaction of Mepacrine Dihydrochloride with Ethanethiol.—(*a*) In ethanolic pyridine. Mepacrine dihydrochloride (1 g.) in ethanolic pyridine (250 ml., 25 : 1 v/v) was treated with ethanethiol (2·5 ml.) and kept in a stoppered flask for 7 days at room temperature. The solvent and excess ethanethiol were removed *in vacuo*, the viscous residue dissolved in benzene, and the solution extracted successively with 5% sodium hydroxide, 20% aqueous acetic acid, and water. The benzene layer was concentrated, dried ($CaCl_2$), and chromatographed on alumina (eluent benzene). The major yellow band yielded 6-chloro-9-ethylthio-2-methoxyacridine (IV; R = Et) (0·56 g., 94%), m. p. 121°. After recrystallisation from light petroleum, the yellow needles had m. p. and mixed m. p. 124·5° (lit., 125°) (Found: C, 63·6; H, 4·85; N, 4·75. Calc. for $C_{16}H_{14}ClNOS$: C, 63·3; H, 4·6; N, 4·6%). The i.r. spectrum was identical with that of a sample prepared by the method of Kitani.[23]

(*b*) In ethanol. The reaction was carried out as above, but with ethanol (250 ml.) as solvent. Yield of the ethylthioacridine (IV; R = Et) after 7 days, 0·3 g., 50%.

(*c*) In pyridine. A saturated solution of mepacrine dihydrochloride in pyridine (150 ml.) was treated with ethanethiol (2·5 ml.). After 4 days at room temperature, the solvent was removed *in vacuo* and the residue extracted between 20% sodium hydroxide and benzene. The benzene layer was concentrated and chromatographed on alumina (eluent benzene), affording the ethylthioacridine (IV; R = Et), estimated yield, 50%; identified by mixed m. p. and by comparison of its i.r. spectrum with that of an authentic sample. Unchanged mepacrine was recovered from the column by elution with chloroform.

Reaction of Mepacrine Free Base with Ethanethiol.—Mepacrine free base (1·95 g.) in solvent (100 ml.) was treated with ethanethiol (2·5 ml.) and the solution kept for 7 days at room temperature. The ethylthioacridine (IV; R = Et) was isolated as described for the reaction between mepacrine dihydrochloride and ethanethiol in ethanolic pyridine. Solvents and yields: (*a*) pyridine; 17 mg. (1%): (*b*) ethanol–pyridine (1 : 1, v/v); 21 mg. (1·5%): (*c*) acetic acid; 22 mg. (1·5%). In (*c*), the 7 day-old reaction mixture was treated with water (400 ml.) and extracted with benzene. The benzene layer was then worked up as described above.

Reaction of Mepacrine Dihydrochloride with Thiophenol.—(*a*) Mepacrine dihydrochloride (1 g.) in ethanolic pyridine (260 ml., 25 : 1 v/v) was treated with thiophenol (2·5 ml.) and the solution kept in a well-stoppered flask for 40 days at room temperature. The reaction mixture was concentrated *in vacuo* to a syrup, which was taken up in benzene and extracted successively with 5% sodium hydroxide, 20% aqueous acetic acid, and water. The benzene layer was dried ($CaCl_2$) and chromatographed on alumina (eluent benzene). Diphenyldisulphide (1·8 g.), m. p. 50—55°, which had an i.r. spectrum identical with that of an authentic sample, m. p. 60—61°, was eluted first. The major yellow band afforded crude 6-chloro-2-methoxy-9-phenylthioacridine (IV; R = Ph) (0·48 g., 70%), characterised by comparison of its i.r. spectrum with that of a sample prepared by the method of Kitani.[23] Further purification of the rude product was frequently difficult, and was best effected, with loss of yield, by partitioning between dilute hydrochloric acid and benzene. The benzene was evaporated and the residue recrystallised successively from light petroleum and ethanol. The fluffy yellow crystals had m. p. 154—155° (lit., 160°), not depressed by addition of authentic material.

(*b*) A saturated solution of mepacrine dihydrochloride in pyridine (100 ml., containing *ca.* 30 mg. of the dihydrochloride) was treated with thiophenol (2·5 ml.) and set aside for 40 days at room temperature. Thin-layer chromatography then showed the yield of the phenylthioacridine (IV; R = Ph) to be 60—80%.

[32] I. Smith, "Chromatographic and Electrophoretic Techniques," Heinemann, London, 2nd edn., 1960, p. 98.

Reaction of Mepacrine Free Base with Thiophenol.—(*a*) Mepacrine free base (1·05 g.) in ether–acetic acid (80 ml., 3 : 1) was treated with thiophenol (0·5 ml.) and the solution set aside in a well-stoppered flask for 20 days at room temperature. Water (80 ml.) and benzene were added. The organic layer was then further extracted with dilute sodium hydroxide and washed well with water. Evaporation of the solvent and recrystallisation of the residue from light petroleum yielded the phenylthioacridine (IV; R = Ph) (0·24 g., 28%), m. p. 157—158°. Further purification by chromatography on alumina and recrystallisation from light petroleum afforded yellow needles, m. p. and mixed m. p. 161° (lit.,[23] 160°).

(*b*) Mepacrine free base (0·79 g.) in pyridine (100 ml.) or ethanolic pyridine (250 ml., 1 : 1) was treated with thiophenol (2·5 ml.) and examined after 40 days at room temperature by thin-layer chromatography. Only traces of the phenylthioacridine (IV; R = Ph) could be detected.

9-*Carboxymethylthio*-6-*chloro*-2-*methoxyacridine* (IV; R = $CH_2 \cdot CO_2H$).—(*a*) A mixture of 6-chloro-2-methoxyacridan-9-thione (VI) (2·55 g.) and chloroacetic acid (1·0 g.) in dioxan (200 ml.) and triethylamine (50 ml.) was heated under reflux for 7 hr. The filtered solution was concentrated *in vacuo* and partitioned between benzene and 0·4M-sodium carbonate. Acidification of the alkaline layer yielded an amorphous precipitate of the *acid* (IV; R = $CH_2 \cdot CO_2H$) (1·77 g., 57%), m. p. 222° (decomp.). After two recrystallisations from acetic acid and one from aqueous dioxan, the acid was obtained as yellow-brown crystals, m. p. 213° (decomp.) (Found: C, 57·7; H, 3·65; N, 4·25. $C_{16}H_{12}ClNO_3S$ requires C, 57·55; H, 3·65; N, 4·2%), $\nu_{max.}$ 1712 cm.$^{-1}$ (carboxyl C=O).

(*b*) 6,9-Dichloro-2-methoxyacridine (1·03 g.), thioglycollic acid (0·5 g.), and phenol (4 g.) were stirred on the water-bath for 2 hr. The cooled mixture was poured into ether, and the solid which separated washed well with ethenol, and reprecipitated from dilute sodium hydroxide solution with acetic acid. The acid (IV; R = $CH_2 \cdot CO_2H$) (0·8 g., 58%) was reprecipitated once more and recrystallised from ethoxyethanol, yielding a micro-crystalline sample, m. p. 224° (decomp.), whose i.r. spectrum was identical with that of the product analysed above.

Reaction of Mepacrine Dihydrochloride with Thioglycollic Acid.—(*a*) Mepacrine dihydrochloride (3·32 g.) in ethanolic pyridine (225 ml., 8 : 1) was treated with thioglycollic acid (10 ml.) and set aside for 7 days at room temperature. The precipitate was filtered off and reprecipitated from 0·4M-sodium carbonate with dilute hydrochloric acid, yielding an amorphous sample of the carboxymethylthioacridine (IV; R = $CH_2 \cdot CO_2H$) (1·12 g., 51%), m. p. 222° (decomp.), whose i.r. spectrum was identical with those of the products above.

(*b*) A solution of mepacrine dihydrochloride (3·09 g.), thioglycollic acid (10 ml.), and triethylamine (25 ml.) in ethanol (170 ml.) was set aside at room temperature for a month. The mixture was filtered, and the filtrate concentrated and then partitioned between a large volume of benzene and 0·4M-sodium carbonate. The thione (VI) (0·13 g.) separated from the benzene layer on standing. Recrystallisation from ethanol afforded red needles, m. p. and mixed m. p. 260° (lit.,[23] 258°).

Reaction of Mepacrine Free Base with Thioglycollic Acid.—Mepacrine free base (3·16 g.) in acetic acid (20 ml.) was treated with thioglycollic acid (2 ml.) and set aside for 4 days at room temperature. Water (30 ml.) was added, and the precipitate collected and redissolved in 0·4M-sodium carbonate. The solution was extracted with benzene and acidified with dilute hydrochloric acid, yielding the acid (IV; R = $CH_2 \cdot CO_2H$) (2·11 g., 80%). After washing with hot ethanol, acetone, and ether, the yellow amorphous product had m. p. 219° (decomp.).

Degradation of Mepacrine by Hydrogen Sulphide in Acetic Acid.—Passage of hydrogen sulphide through a solution of mepacrine free base (2·36 g.) in acetic acid (100 ml.) at 80° for 8 hr. gave a red solution, which on standing at room temperature deposited red crystals of the thione (VI) (0·9 g., 55%), m. p. and mixed m. p. 263°.

Reaction between Mepacrine and Cysteine.—Mepacrine dihydrochloride (3·0 g.) and L-cysteine hydrochloride (0·47 g.) were dissolved in 0·07M-phosphate buffer (80 ml.), pH 7·1, and set aside under nitrogen at 37°. After 11 days the NS-*bis* (6-*chloro*-2-*methoxy*-9-*acridinyl*)-L-*cysteine* (IX) (1·37 g., 73%) was filtered off and washed well with buffer, water, warm ethanol, and acetone. Further purification was effected by dissolution in ethanol–conc. ammonia (300 ml., 4 : 1), followed by addition of acetic acid (60 ml.). Within a few minutes amorphous bisacridinylcysteine (IX) separated, which after a further reprecipitation and washing with ethanol had m. p. 175—176° (decomp.) and $[\alpha]_D^{20}$ +12° (*c.* 1·05) (ethanol–conc. ammonia, 4 : 1) (Found:

C, 57·95; H, 4·3; N, 6·5. $C_{31}H_{23}Cl_2N_3O_4S\cdot 2H_2O$ requires C, 58·1; H, 4·2; N, 6·55%), $\nu_{max.}$ 3370 (broad, H_2O), 3220 (NH), 2930, 2820, 1628, 1582, 1537, and 1502 cm.$^{-1}$, $\lambda_{max.}$ (ethanol–conc. ammonia, 4 : 1) 226, 265, 347, 364, and 403 mμ (ε 47,200, 111,000, 5960, 9200, and 11,700). A sample dried (P_2O_5) *in vacuo* regained weight rapidly on exposure to the atmosphere. In some preparations, changes in the degree of hydration were associated with colour changes from yellow (hydrated) to orange (anhydrous).

A portion of the filtrate from the above reaction mixture was chromatographed in solvent system (A). The spots were located by (*a*) fluorescence, R_F 0·37 (w), 0·56 (w), 0·83 (mepacrine), and 1·0; (*b*) ninhydrin, R_F 0·09 (cysteine), 0·14 and 0·22—0·34 ("salt carried" material); (*c*) iodine–azide, R_F 0·09 (cysteine) and 0·22—0·34 ("salt carried" material).

Degradation of NS-*Bis*(6-*chloro*-2-*methoxy*-9-*acridinyl*)-L-*cysteine in Acetic Acid.*—The bisacridinylcysteine (IX) (0·75 g.) in acetic acid (50 ml.) was heated under reflux for 1 hr., and the resulting solution set aside at room temperature overnight. The solid which separated was filtered off and extracted with acetone.

(*a*) Acetone extract. Evaporation of the acetone left red-gold crystals (0·14 g.) of the thione (VI), which after recrystallisation from ethanol had m. p. and mixed m. p. 261—262°.

(*b*) Residual solid. The acetone-washed solid (0·26 g.) on recrystallisation from dioxan yielded fine yellow crystals of 9-*acetylamino*-6-*chloro*-2-*methoxyacridine acetate* (X; R = CO·Me) m. p. 275° (Found: C, 58·3; H, 4·85; Cl, 9·85; N, 7·55. $C_{18}H_{17}ClN_2O_4\cdot\frac{1}{2}H_2O$ requires C, 58·45; H, 4·9; Cl, 9·6; N, 7·6%), $\nu_{max.}$ 3450—3180 (broad shoulder, H_2O and NH) and 1690 cm.$^{-1}$ (N-acetyl C=O), $\lambda_{max.}$ 275·5, 326, 341, 412, and 434·5 mμ (ε 52,100, 3770, 5920, 7030, and 6360).

9-*Acetylamino*-6-*chloro*-2-*methoxyacridine Acetate* (X; R = CO·Me).—This was prepared following the method of Wilkinson and Finar.[25] To a suspension 9-amino-6-chloro-2-methoxyacridine (X; R = H) (1·0 g., prepared by the method of King, Beer, and Whaley [33]) in pyridine (8 ml.), acetic anhyride (0·52 ml.) was added. The mixture was stirred on the water-bath for 3 hr. and, after cooling, poured into benzene (50 ml.). The acetylaminoacridine acetate (X; R = CO·Me) was filtered off and recrystallised successively from acetic acid and dioxan, when it had m. p. 277°. A sample did not depress the m. p. of the degradation product from the previous experiment and the i.r. spectra were identical.

Pyrolysis of 9-*Acetylamino*-6-*chloro*-2-*methoxyacridine Acetate.*—The acetylaminoacridine (X; R = CO·Me) was heated at 180° *in vacuo* (0·1 mm.) in a sublimation tube. The sublimate, 9-amino-6-chloro-2-methoxyacridine (X; R = H), had m. p. and mixed m. p. 278°. Its i.r. spectrum was identical with that of an authentic specimen.

Degradation of NS-*Bis*(6-*chloro*-2-*methoxy*-9-*acridinyl*)-L-*cysteine by Hydrogen Sulphide.*—The bisacridinylcysteine (IX) (20 mg.) was suspended in 0·8M-sodium carbonate–ethanol (25 ml., 1 : 1). Hydrogen sulphide gas was passed through the mixture for 5 hr., and the suspension was left overnight. Water was then added, and the solid remaining removed by centrifugation. The supernatant was partly desalted, electrolytically, and portions chromatographed against an authentic sample of L-cysteine hydrochloride, which had been similarly partly desalted. (*a*), Solvent system (A): (i), reaction mixture, ninhydrin-positive, R_F 0·10, 0·24 (w), and 0·32, and iodine–azide-positive, R_F 0·10 and 0·32; (ii), cysteine, ninhydrin-positive, R_F 0·10 and 0·32, and iodine–azide-positive, R_F 0·10 and 0·32. (*b*), Solvent system (B): (i), reaction mixture, ninhydrin-positive, R_F 0·11 (w) and 0·23; (ii), cysteine, ninhydrin-positive, R_F 0·11 (w) and 0·23. The double spot for cysteine arises from the presence of inorganic salts. The spots from the reaction mixture were, in general, weak.

In degradation experiments using ethanol–conc. ammonia as solvent and larger amounts of the bisacridinylcysteine (IX), cysteine was again the major water-soluble product, but other weak ninhydrin-positive spots were observed (solvent system (A), R_F 0·17, 0·24, and 0·45). The precipitated product was identified as the thione (VI).

N-(6-*Chloro*-2-*methoxy*-9-*acridinyl*)-DL-α-*alanine* (XI; R = Me).—(*a*) To 6-chloro-2-methoxy-9-phenoxyacridine [34] (2·0 g.) in phenol (4·0 g.), DL-α-alanine (0·53 g.) was added, and the mixture stirred for 5 hr. on an oil-bath at 120°. The viscous product was cooled and poured into ether. The yellow solid which separated was filtered off, dissolved in dilute sodium hydroxide and extracted with chloroform until the organic layer showed no blue fluorescence. N-(6-*chloro*-2-*methoxy*-9-*acridinyl*)-DL-α-*alanine* (XI; R = Me) (1·14 g., 57%) was recovered from the alkali layer by addition of 20% acetic acid. Further purification was effected by twice

[33] F. E. King, R. J. S. Beer, and S. G. Whaley, *J.*, 1946, 92.
[34] N. S. Drosdow and S. A. Skripizyna, *Chem. Zentral.*, 1937, **108**, *II*, 3604.

repeating the extraction and reprecipitation. Crystallisation from acetic acid yielded the acetate, m. p. 230° (decomp.), $\nu_{max.}$ near 1720 cm.$^{-1}$ (carboxyl C=O). Heating the acetate *in vacuo* (0·3 mm.) at 90° afforded the *N*-acridinylamino-acid (XI; R = Me), m. p. 222° (decomp.) (Found: C, 60·3; H, 4·4; N, 7·95. $C_{17}H_{15}ClN_2O_3 \cdot \frac{1}{2}H_2O$ requires C, 60·1; H, 4·7; N, 8·25%), $\nu_{max.}$ 3420 (H_2O) and 3210 cm.$^{-1}$(NH).

(*b*) 6,9-Dichloro-2-methoxyacridine (1·0 g.) was dissolved in phenol (5·0 g.) at 95°. DL-α-Alanaine (0·33 g.) was added, and the mixture stirred for 1 hr. The viscous solution was allowed to cool, and poured into ether. The solid formed was filtered off and reprecipitated from ethanol–conc. ammonia (1 : 1) with 20% acetic acid, yielding the *N*-acridinylamino-acid (XI; R = Me) (0·62 g., 51%), m. p. 225° (decomp.). The i.r. spectrum was identical with that of the sample above.

A suspension of the *N*-acridinyl-α-alanine (XI; R = Me) in ethanol–pyridine (4 : 1) was treated with hydrogen sulphide for 3 hr. The solid material was centrifuged off and a portion of the supernatant chromatographed in solvent system (A) against α-alanine. Control and experiment each showed one ninhydrin-positive spot at R_F 0·25.

N-(6-*Chloro*-2-*methoxy*-9-*acridinyl*)-DL-*methionine* (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$).—(*a*) To 6,9-dichloro-2-methoxyacridine (2·0 g.) dissolved in phenol (10 g.), DL-methionine (1·07 g.) was added, and the mixture stirred on the water-bath for 40 min. The reaction mixture was poured into ether, and the solid which separated extracted with 2·5% sodium hydroxide–ethanol (30 ml., 1 : 1). On careful addition of 20% acetic acid, the *N-acridinylmethionine* (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$) separated as fine crystals (0·92 g., 33%). After repeating this process, the yellow crystals had m. p. 182° (decomp.) (Found: C, 56·95; H, 5·3; N, 6·7. $C_{19}H_{19}ClN_2O_3S \cdot \frac{1}{2}H_2O$ requires C, 57·0; H, 5·0; N, 7·0%), $\nu_{max.}$ 3320 cm.$^{-1}$ (broad shoulder, H_2O).

(*b*) A mixture of 6-chloro-2-methoxy-9-phenoxyacridine (2·0 g.), DL-methionine (0·89 g.), and phenol (8 g.) was stirred at 105° for 5 hr. The viscous product was poured into ether, and the solid which separated reprecipitated from 2·5% sodium hydroxide with 20% acetic acid, yielding the crude acridinylmethionine (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$) (2·11 g., 89%), m. p. 196° (decomp.). This was purified by extracting a solution of the compound in dilute sodium hydroxide with chloroform until the organic layer showed no blue fluorescence. The amino-acid was reprecipitated by the addition of acetic acid. Successive recrystallisations from aqueous acetic acid gave m. p. 185—186, 182, and 182—183° (all decomp.). The i.r. spectrum of the final product was identical with that of the product from (*a*).

Degradation of the *N*-acridinylmethionine (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$) with hydrogen sulphide, as described for the *N*-acridinyl-α-alanine (XI; R = Me) above, followed by chromatography in solvent system (A), yielded one ninhydrin-positive spot, having the same R_F (0·55) as a control of DL-methionine.

Reaction of Mepacrine with Amino-acids.—Equivalent amounts of (*a*) glycine (0·45 g.), (*b*) DL-α-alanine (0·53 g.), and (*c*) DL-methionine (0·88 g.) were added to mepacrine dihydrochloride (3·0 g.) in 0·07M-phosphate buffer (80 ml.), pH 6·9, and the solutions were left at 37° under nitrogen. After 10 days the precipitates were filtered off, washed, and dried. The filtrates were made alkaline with sodium hydroxide and extracted with chloroform. Acidification of the alakaline layer yielded the appropriate *N*-acridinylamino-acid (XI). All products were identified by comparison of their i.r. spectra with those of authentic samples.

(*a*) The yellow precipitate (0·18 g.) was impure *N*-(6-chloro-2-methoxy-9-acridinyl)glycine (XI; R = H). A second crop was obtained from the filtrate, giving in all 0·19 g. (10%). An authentic sample of the product was prepared by the method of Burckhalter *et al.*[35]

(*b*) The yellow precipitate (0·04 g.) was the very impure *N*-acridinyl-α-alanine (XI; R = Me). The filtrate yielded a further 5 mg. The total yield of pure material (0·02—0·03 g.) was 1—1½%.

(*c*) The yellow precipitate (0·01 g.) was 6-chloro-2-methoxyacridone m. p. >360° (lit.,[36] >360°). The filtrate afforded the *N*-acridinylmethionine (XI; R = $CH_2 \cdot CH_2 \cdot S \cdot CH_3$) (0·05 g., 2%), m. p. 188° (decomp.).

Degradation of NS-*Bis*(6-*chloro*-2-*methoxy*-9-*acridinyl*)-L-*cysteine* (IX) *in Basic Solution.*—A suspension of the bisacridinylcysteine (IX) (0·99 g.) in triethylamine (30 ml.) and dimethylformamide (60 ml.) was heated under reflux for 1½ hr., by which time the solid had dissolved.

[35] J. H. Burckhalter, E. M. Jones, W. F. Holcomb, and L. A. Sweet, *J. Amer. Chem. Soc.*, 1943, **65**, 2012.

[36] G. I. Braz, *Chem. Abs.*, 1942, **36**, 4122.

The mixture was concentrated to small volume, a large volume of ether added, and the solid which separated filtered off. This substance was not identified. The filtrate, on standing at 4°, deposited red crystals of the thione (VI) (0·22 g.), which after recrystallisation from ethanol had m. p. 255° (lit.,[23] 258°).

Degradation of N-(6-*Chloro*-2-*methoxy*-9-*acridinyl*)*glycine* (XI; R = H) *in Basic Solution.*—A suspension of the *N*-acridinylglycine (XII; R = H) (0·36 g.) in triethylamine (12 ml.) and dimethylformamide (24 ml.) was heated under reflux for 24 hr., by which time all the solid had dissolved. The mixture was poured into water, and the yellow solid which separated filtered off. After four recrystallisations from aqueous dioxan (1 : 1), the 6-chloro-2-methoxy-9-methylaminoacridine (X; R = Me) had m. p. 135—137° (Found: C, 65·35; H, 4·85; N, 10·0. Calc. for $C_{15}H_{13}ClN_2O$: C, 66·1; H, 4·75; N, 10·3%). Sublimation *in vacuo* raised the m. p. to 175° (lit.,[23] 174°) (Found: N, 10·35%), $\lambda_{max.}$ (the same for both isomers, ethanol) 225, 268, 283, 327, 342·5, 360, 419, and 438 mμ (ε 26,700, 46,800, 53,700, 1540, 2780, 2880, 8040, and 6480). A sample was also prepared using the method of Dupré and Robinson.[16] 6-Chloro-2-methoxy-9-phenoxyacridine (0·7 g.), methylamine hydrochloride (0·13 g.), and phenol (3·5 g.) were heated at 110° with stirring for 1 hr. The mixture was allowed to cool, and poured into ether. The yellow solid which separated was washed with dilute ammonium hydroxide, yielding crude methylaminoacridine (X; R = Me) (0·53 g., 92%), m. p. 135—140°. After recrystallisation from aqueous dioxan (1 : 1) it had m. p. and mixed m. p. 176° and an i.r. spectrum identical with that of the isomer m. p. 175° above.

Degradation of N-(6-*Chloro*-2-*methoxy*-9-*acridinyl*)-DL-α-*alanine* (XI; R = Me) *in Basic Solution.*—This was carried out in the same manner as for the *N*-acridinylglycine (XI; R = H). The 6-chloro-9-ethylamino-2-methoxyacridine (X; R = Et) produced was identified by comparison of its i.r. spectrum with that of a sample synthesised by the method described above.

Reaction of Mepacrine with Glutathione (*GSH*).—A solution of mepacrine dihydrochloride (0·39 g.) and GSH (0·1 g.) in 0·07M-phosphate buffer (10 ml.), pH 7·1, was left at 37° under nitrogen for 6 weeks. The red crystalline solid (0·13 g.) which formed was filtered off.

(*a*) Filtrate. The filtrate was desalted electrolytically, during which a yellow precipitate separated. A portion of the desalted filtrate, mixed with an ethanolic solution of the precipitate, was chromatographed against GSH–oxidised GSH (GSSG) (obtained from an aqueous solution of GSH which had been standing for some time exposed to air) and 4-diethylamino-1-methylbutylamine (III) (obtained by degrading mepacrine free base in ethanolic pyridine with hydrogen sulphide. The thione (VI) was removed by centrifugation, the supernatant evaporated to dryness, and the residue extracted with water). Solvent system (A): (i), Filtrate. Fluorescence, R_F 0·52, 0·80, and 0·92; ninhydrin-positive, R_F 0·05, 0·19, and 0·26; iodine–azide-positive, R_F 0·05 and 0·19. (ii), GSH–GSSG. Fluorescence, nil; ninhydrin-positive, R_F 0·05 and 0·19; iodine–azide-positive, R_F 0·05 and 0·19. (iii), Diamine (III). Fluorescence, nil; ninhydrin-positive, R_F 0·26; iodine–azide-positive, nil. Solvent system (C). The desalted filtrate had been standing in air for a day when this chromatogram was run. (i), Filtrate. Ninhydrin-positive, R_F 0·32 and 0·92. (ii), GSH–GSSG. Ninhydrin-positive, R_F 0·32 and 0·53. (iii), Diamine (III). Ninhydrin-positive, R_F 0·92.

(*b*) Red crystalline solid. The substance was washed well with water, and the last portion of the yellow washings, which were ninhydrin negative, was partitioned between sodium hydroxide and ether. The ethereal layer showed, on thin-layer chromatography, one yellow–green fluorescent spot, with the same R_F as a control of mepacrine base. After drying, the red solid had m. p. 178° (decomp.).

Degradation of the Mepacrine–GSH Reaction Product by Hydrogen Sulphide.—The mepacrine–GSH reaction product (30 mg.), suspended in ethanol–pyridine (2·5 ml., 4 : 1), was treated with hydrogen sulphide for 5 hr. The solid material was centrifuged off. An aqueous extract of the solid showed the same pattern of ninhydrin-positive spots on thin-layer chromatography as the ethanol–pyridine supernatant. The aqueous extract was chromatographed against GSH–GSSG and the diamine (III) (both controls prepared as described above). Solvent system (A). Run on thin-layer plates, fixed phase cellulose. (i), Reaction product. Ninhydrin-positive, R_F 0·14 (s), 0·18, 0·24 (w), 0·46, and 0·53 (vw); iodine–azide-positive, R_F 0·14 (s), 0·18, and 0·24 (w). (ii), GSH–GSSG. Ninhydrin-positive, R_F 0·14, 0·18 (w), and 0·32; iodine–azide-positive, R_F 0·14, 0·18(w), and 0·32. (iii), Diamine (III). Ninhydrin-positive, R_F 0·46 and 0·53 (w); iodine–azide-positive, nil. Solvent system (C). All spots detected with ninhydrin. (i), Reaction product, R_F 0·37, 0·46 (vw), and 0·95. (ii), GSH–GSSG, R_F 0·37

and 0·61. (iii), Diamine (III), R_F 0·95. Solvent system (D). All spots detected with ninhydrin. (i), Reaction product. R_F 0·11 and 0·75. (ii), GSH–GSSG, R_F 0·11 and 0·41. (iii), Diamine (III), R_F 0·75.

N-*Toluene*-p-*sulphonyl*-S-(6-*chloro*-2-*methoxy*-9-*acridinyl*)-2-*mercaptoethylamine* (XIII; R = $SO_2 \cdot C_6H_4 \cdot CH_3$).—6-Chloro-2-methoxyacridan-9-thione (VI) (1·0 g.) was added to a solution of metallic sodium (0·095 g.) in ethanol (70 ml.), and the temperature raised to just below boiling. *N*-Toluene-*p*-sulphonyl-2-chloroethylamine [37] (0·85 g.) in ethanol (50 ml.) was added dropwise to the stirred solution over a period of 1 hr. After further heating under reflux for 15 min. the reaction mixture was filtered hot. Yellow crystals of N-*toluene*-p-*sulphonyl*-S-(6-*chloro*-2-*methoxy*-9-*acridinyl*)-2-*mercaptoethylamine* (XIII; R = $SO_2 \cdot C_6H_4 \cdot CH_3$) (1·33 g., 78%), m. p. 198—201°, separated from the filtrate on cooling. Further recrystallisation from benzene raised the m. p. to 203° (Found: C, 58·15; H, 4·65; N, 5·85. $C_{23}H_{21}ClN_2O_3S_2$ requires C, 58·4; H, 4·45; N, 5·95%), $\lambda_{max.}$ (ethanol) 226·5, 267·5, 347, 362, 396, and 417 mμ (ε 29,400, 104,000, 5560, 8540, 6980, and 7130). Compare 6-chloro-9-ethylthio-2-methoxyacridine (IV; R = Et), $\lambda_{max.}$ (ethanol) 224, 267, 350 (shoulder), 362, 394·5, and 414 mμ (ε 20,000, 55,500, 5430, 8300, 7250, and 7340) and 6-chloro-2-methoxyacridan-9-thione (VI), $\lambda_{max.}$ (ethanol) 245, 294, 305·5, 356·5, 436·5, and 493 mμ (ε 51,000, 19,800, 19,000, 4640, 5470, 15,500, and 25,400).

N-*Benzyloxycarbonyl*-S-(6-*chloro*-2-*methoxy*-9-*acridinyl*)-2-*mercaptoethylamine* (XIII; R = $CO \cdot O \cdot CH_2 \cdot C_6H_5$).—A solution of the thione (VI) (1·5 g.) and metallic sodium (0·15 g.) in ethanol (60 ml.) was treated with *N*-benzyloxycarbonyl-2-bromoethylamine (1·55 g., see below) in ethanol (10 ml.), and the resulting solution heated under reflux for 1 hr. The solid which separated on cooling was filtered off and washed with ethanol and water. The impure N-*benzyloxycarbonyl derivative* (2·01 g., 82%), m. p. 150—151°, was recrystallised successively from methanol, ethoxy-ethanol, and light petroleum–tetrahydrofuran (1 : 1), until thin-layer chromatography showed one spot only. The feathery, pale yellow crystals had m. p. 158° (Found: C, 63·7; H, 4·7; Cl, 7·7; N, 5·85. $C_{24}H_{21}ClN_2O_3S$ requires C, 63·65; H, 4·65; Cl, 7·85; N, 6·2%).

The *N*-benzyloxycarbonyl-2-bromoethylamine was prepared from a solution of benzyloxycarbonyl chloride in toluene [38] and 2-bromoethylamine following the method of Lindley.[39]

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