Some Properties of Acridane and 2-Chloro-7-methoxyacridane: their Possible Relationship to Excretion Products of Mepacrine*

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The separation of basic excretion products of mepacrine (2-chloro-5-(4'-N:N-diethylamino-1-methyl-butylamino)-7-methoxyacridine dihydrochloride) from human urine has been reported previously (King, Gilchrist & Tárnoky, 1946). A number of degradation products appeared, on spectrographic evidence, to be diphenylamine derivatives, but the possibility of their being acridanes could not be excluded. The latter are electronic analogues of diphenylamines, resonance in the centre nucleus having been destroyed. They are known to be similar in their molecular refractions (Auwers & Kraul, 1925*a, b*) and may be expected to have similar absorption spectra. Moreover, the change from mepacrine to an acridane derivative is smaller than would be the rupture of the pyridine ring in acridine.

On the other hand, the relatively low stability of acridanes made their presence in the urine or the urine extracts unlikely. Furthermore, our method of separation involved an extraction with hydrochloric acid (0.5*N*) in which acridanes are generally considered to be insoluble. On neither of these two points, however, was quantitative data available.

The aim of this work was to obtain such data regarding the stability of two representative acridanes and their solubility in 0.5*N*-hydrochloric acid. Acridane was chosen as the most readily accessible compound of its class, and 2-chloro-7-methoxyacridane as one closely related to mepacrine. The ultraviolet absorption spectra of these two compounds were also determined. They are reproduced here for comparison with the spectra of diphenylamines and of mepacrine degradation products published in the previous paper.

EXPERIMENTAL

Acridane. 5-Chloroacridine (5 g.) in alkaline aqueous-ethanolic-benzene solution was hydrogenated in the presence of a large excess of Raney Ni (3.5 g.), as described by Albert & Willis (1946). H_2 uptake: 1180 ml. at 24° (theory for 4H: 1100 ml. at 15°). Air was admitted, ethanol (100 ml.) added, and the mixture heated on the steam bath in an atmosphere of N_2 , and quickly filtered.

On evaporation, the filtrate yielded a crystalline residue which was heated with 2*N*-HCl on the steam bath for

10 min. The acid-insoluble solid, separated by filtration, was twice recrystallized from hot acetone-water. Yield: 3.5 g., m.p. 168–170° (corr.). Albert & Willis (1946) give m.p. 169–170°.

The identity of this substance was further confirmed by comparison with a specimen of acridane obtained by Kane (1950) by controlled-potential electrolytic reduction of acridine. This specimen melted at 168–169°; admixture with the acridane, prepared as described above, gave m.p. 167–169° (both m.p. uncorr.).

2-Chloro-7-methoxyacridane. 2,5-Dichloro-7-methoxyacridine (6.5 g.) was hydrogenated in the same way as above, but more benzene (50 ml.) and ethanol (200 ml.) were used. The residue from the filtered hydrogenation product was powdered, heated in 200 ml. 2*N*-HCl at 80° for 10 min. and separated by filtration. It was heated in acetone solution with animal charcoal, filtered and recrystallized from aqueous acetone. One more acid extraction followed by one recrystallization from aqueous acetone and two from benzene-light petroleum yielded the pure 2-chloro-7-methoxyacridane as a pale yellow microcrystalline powder, m.p. 185–186° (corr.) in a sealed tube. (Found: N, 5.9; Cl, 14.9. Calc. for $C_{14}H_{12}ONCl$: N, 5.7; Cl, 14.5%.)

This compound has been obtained by a different method by Hall & Turner (1945) who give m.p. 187–188°.

Stability of the acridane in aqueous-ethanolic solutions at room temperature

Approximately 0.01*M*-solutions of each acridane were prepared in 90% ethanol–10% water solution, and in 90% ethanolic–10% aqueous NaOH and HCl of 0.01*N*- and 0.1*N*-final concentrations respectively. These solutions were allowed to stand at room temperature in dark, loosely stoppered bottles, for a period between November and January. The amount of unchanged acridane was estimated, by the method described below, in portions withdrawn periodically from the solutions.

Estimation of acridanes

A portion of the solution (20 ml.) was diluted with ethanol (10 ml.). Na acetate (anhydrous 0.3 g.) and 2*N*-HCl (0.3 ml. to the neutral, 0.4 ml. to the alkaline and 0.1 ml. to the acid solutions) were added to give a pH 5.6–5.8; this was followed by 0.1*N*- I_2 (10 ml.). The solution was allowed to stand in a glass-stoppered flask at room temperature for 4 days, by which time oxidation of the acridane by iodine was complete. The excess I_2 was determined by means of 0.05*N*- $Na_2S_2O_3$ solution, using a starch phenol red indicator.

*Solubility of acridane in aqueous 0.5*N*-hydrochloric acid*

(i) 'Hot.' An excess of acridane was added to 0.5*N*-HCl at 80° and the mixture was shaken mechanically and allowed to cool. Room temperature (21°) was reached in

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1 hr. A weighed amount (about 3 g.) of the saturated solution, obtained by filtering into a 5 ml. weight pipette, was washed into a flask by means of ethanol; Na acetate (anhydrous, 5 g.) was added, followed by 0.004 N- I_2 (30 ml.). The solution was allowed to stand at room temperature for 10 days, and the excess I was determined by titrating with 0.005 N- $Na_2S_2O_3$. Solubility of acridane at 21° in 0.5 N-HCl: 42 mg./1000 g. solution.

(ii) 'Cold.' Ice-cold 0.5 N-HCl was shaken mechanically with acridane until room temperature was reached. The same procedure as in (i) gave the solubility at 21° as 55 mg./1000 g. solution.

Solubility of 2-chloro-7-methoxyacridane in aqueous 0.5 N-hydrochloric acid

Determinations (i) and (ii) were attempted as above. All the added I_2 (30 ml. of 0.004 N-solution) was used up in both experiments.

Spectrophotometry

Absorption spectra in the ultraviolet and visible range were recorded by Mr W. R. Weedon using a Beckman spectrophotometer. The acridanes were examined in ethanolic solution and also in 50 % ethanolic-50 % aqueous solutions containing 0.5 N-HCl or 0.5 N-NaOH (final concentrations), and at pH 7.6 using Palitzsch boric acid borax buffer (Britton, 1942). The approximate ages of the solutions at the commencement of the spectrum determinations were as follows: acridane at pH 7.6, 3 hr.; acridane in NaOH, 1 hr.; acridane in HCl, 20 min.; all solutions of 2-chloro-7-methoxyacridane, 15 min.

RESULTS

The stability of acridane solutions

The rates of decomposition of six solutions are shown in Table 1. The iodine titres have been recalculated to refer to exactly 0.01 M-solutions of acridanes. These results are expressed as percentages of unchanged acridane or 2-chloro-7-methoxyacridane, '100 %' referring to an unchanged acridane solution of 0.01 M-strength.

Absorption spectra

Acridane (Fig. 1). The spectrum of this compound in ethanol solution shows a single wide absorption band with λ_{max} at 2900 Å. and $\epsilon \times 10^{-3} = 13.68$ (or $\log \epsilon = 4.136$). These values agree well with the data obtained by Blout & Corley (1947) who record a single peak at 2890 Å. ($\log \epsilon = 4.14$). These workers note the agreement of this curve with that of diphenylamine (Castille, 1926).

The solutions in hydrochloric acid, sodium hydroxide and at pH 7.6 all show the same absorption maximum at 2900 Å. None of these curves, however, are pure acridane spectra. An inflexion at 2500 Å. of the ethanolic solution becomes well marked in sodium hydroxide, while the hydrochloric acid solution shows the sharp absorption band of the acridine spectrum with λ_{max} at 2550 Å. (King *et al.* 1946). The long wave band of acridine (λ_{max} 3550 Å.)

Table 1. *Stability of 0.01 M-acridane and 2-chloro-7-methoxyacridane solutions at room temperature*

Age of solution	Percentage of original acridane content
Solution 1. Acridane in 10 % water-90 % ethanol	
0.5 hr.	104
5	100
23	94
4 days	86
8	70
12	62
20	49
29	30
36	20
50	6
64	7
Solution 2. Acridane in 10 % aqueous-90 % ethanolic 0.01 N-NaOH	
0.5 hr.	102
3.5	101
2 days	92
5	87
8	82
14	78
23	66
30	62
44	54
58	50
Solution 3. Acridane in 10 % aqueous-90 % ethanolic 0.1 N-HCl	
3 hr.	29
24	9
7 days	11
16	0
23	0
Solution 4. 2-Chloro-7-methoxyacridane in 10 % water-90 % ethanol	
0.5 hr.	100
3	93
24	31
3 days	0
16	0
Solution 5. 2-Chloro-7-methoxyacridane in 10 % aqueous-90 % ethanolic 0.01 N-NaOH	
0.5 hr.	85
6	79
28	64
52	53
15 days	22
20	18
26	18
33	18
Solution 6. 2-Chloro-7-methoxyacridane in 10 % aqueous-90 % ethanolic 0.1 N-HCl	
0 hr.	32
24	10
14 days	5
19	3
25	3
32	5

is also well developed, and the solution fluoresces whitish blue. Generally, acridane is seen to decompose faster in acid than in alkaline solution, and

Fig. 1. Spectral absorption curves of acridane solutions. Curve A was determined starting measurements at 2200 A. and proceeding steadily to 4500 A. The $\lambda_{\max}$ at 2550 A. represents approx. 12% conversion to acridine in a solution about 30 min. old; the $\lambda_{\max}$ at 3550 A. corresponds to the presence of approx. 65% acridine about 30 min. later. A, —, 0.5 N-HCl (50% ethanol); B, ---, pH 7.6 (50% ethanol); C, , 0.5 N-NaOH (50% ethanol); D, - · - · - , ethanol.

Fig. 2. Spectral absorption curves of 2-chloro-7-methoxyacridane solutions. Curves A (determined starting from 4800 A. and proceeding to 2200 A.) and C (determined from 2300 A. towards 4200 A.) show more marked changes than the corresponding curves in Fig. 1. A, —, 0.5 N-HCl (50% ethanol); B, ---, pH 7.6 (50% ethanol); C, , 0.5 N-NaOH (50% ethanol); D, - · - · - , ethanol.

least in ethanol. The 'growth' of the peak at 2550 A. would suggest that this band is not part of the acridane spectrum, and that even the ethanol solution, in common with that of Blout & Corley (1947), contains a trace of acridine. This peak does not appear in the spectrum of diphenylamine.

2-Chloro-7-methoxyacridane (Fig. 2). A wide absorption band with $\lambda_{\max}$ at 2920 A. is present in the ethanol, sodium hydroxide and pH 7.6 spectra ($\epsilon \times 10^{-3}$ in ethanol = 15.1 at 2920 A.; $\epsilon \times 10^{-3}$ in sodium hydroxide = 16.7 at 2920 A.) but is not observed in the acid solution within 15 min. In addition to the two bands characteristic of acridine, this solution shows a weak absorption band at 3150 A. and a larger, diffuse band in the 3700–4600 A. region, with $\lambda_{\max}$ at 4200 A. The single absorption peak of 2-chloro-7-methoxyacridane itself is very similar to that of the parent acridane but this spectrum is considerably blurred by the superimposed acridine bands. The chloromethoxy compound changes visibly, more so than acridane. The acid solution is yellow from the start. At 4000 A. the 15 min. old sodium hydroxide solution has $\epsilon \times 10^{-3} = 0.56$; 30 min. later $\epsilon \times 10^{-3} = 1.25$. As in the case of acridane, the ethanolic solution is the most stable, the one in acid the least stable. In both cases, the spectra in hydrochloric acid are included as giving an indication of the instability of these compounds rather than from the point of view of the spectroscopy of pure acridanes.

DISCUSSION

The outstanding characteristic of these two compounds is their instability. This property is demonstrated by the behaviour of their ethanolic solutions at room temperature; it also interferes with the measurement of their acid solubilities and absorption spectra. Under the conditions selected, acridane is more stable than its 2-chloro-7-methoxy-analogue. Both are more stable in alkaline than in acid solution. This is shown by their absorption spectra, which are as much a measure of the instability of these compounds as of their optical properties. Thus simple acridanes are too unstable to survive unchanged in the voided urine or throughout the extraction although stabilized forms (cf. compounds substituted in the 5 and 10 position) might survive. The change undergone by acridane is clearly an oxidation to acridine. In the case of the chloromethoxy compound the spectrum of the corresponding acridine is not available for comparison, and the acid spectrum may indicate some further or additional reaction, but even here the primary change is probably to form 2-chloro-7-methoxy-acridine. While, therefore, the diphenylamine derivatives referred to in the previous paper may have been formed by way of acridanes, it is most unlikely that this latter stage of the reaction took place during the extraction procedure.

On the basis of spectrographic evidence the acridanes are not similar to any of the mepacrine fractions or to the particular diphenylamine derivatives previously examined. A stabilized form (for in-

stance of 5:10-dihydromepacrine) may of course have a substantially different spectrum. It is possible that such a compound would absorb at higher wavelengths than both mepacrine and its degradation products do. The possible formation of a diphenylamine derivative retaining the mepacrine side chain was suggested in the first paper.

The results of the solubility experiments must also be considered in conjunction with the low stability of these compounds. A solution containing about 50 mg./l., although much too dilute for preparative work, would suffice for spectroscopy and, given stability, the correct spectrum in the ultraviolet region could be obtained. The result with acridane, however, suggests that the slowly dissolving compound is simultaneously oxidized into the stable and soluble acridine, with some unchanged acridane present at the time of the solubility determination, possibly as the hydrochloride of the molecular compound between acridine and acridane suggested by Blout & Corley (1947). The unexplained result obtained with the chloromethoxy compound is presumably connected with the additional change observed in the spectrum of its acid solution.

SUMMARY

1. The stability, acid-solubility and ultraviolet absorption spectra of acridane and of 2-chloro-7-methoxyacridane have been measured under selected conditions.

2. The instability of these compounds, especially in acid media, makes measurements of solubility and absorption spectra of doubtful accuracy, and sometimes impossible.

3. Previous findings on the nature of mepacrine degradation products have been compared with those of the present work. The conclusions formerly reached still hold, but acridanes unsubstituted in the 5:10-positions can now be ruled out as excretion products from subjects on mepacrine therapy.

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The Effect of Oxygen and of Cocarboxylase on the Formation of Citrate and α -Ketoglutarate by Pigeon Brain Homogenates

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The fact that vitamin B₁ is necessary for the relief of deficiency signs in the pigeon and for the disappearance of added or accumulated pyruvate *in vitro* and *in vivo* has always been an indirect argument for the decarboxylation of pyruvate to some C₂ fragment before its further oxidation, since aneurin pyrophosphate is concerned essentially with decarboxylation; thus in the animal an oxidative decarboxylation has appeared to be obligatory. In spite of this cogent evidence there has been no proof

that, in fact, such a C₂ fragment is formed, which is clearly an unsatisfactory position. In a recent communication (Coxon, Liébecq & Peters, 1949) it was shown that citrate and α -ketoglutarate are produced during the oxidation of pyruvate by dialysed homogenates of pigeon brain, and that the yields of these compounds are increased greatly when fumarate is added. Though all difficulties in the application of the tricarboxylic acid cycle to brain tissue were not resolved, these findings pointed to the condensation of a derivative of pyruvate, or of pyruvate itself, with a four-carbon dicarboxylic acid along the lines

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