THE IDENTIFICATION AND DETERMINATION OF NITROGENOUS ORGANIC BASES WITH AMMONIUM REINECKATE

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The identification and determination of several important bases as their reineckates is described. The ultra-violet and visible spectra of reineckates were studied and used for the determination of molecular weights and solubilities and for the determination of bases. This paper also records as yet unreported constants of the characteristic mono- and di-reineckate derivatives of several clinically important compounds. A method for the regeneration of the conjugate bases from the reineckates using ion exchange resins is also given.

Many organic bases react with ammonium reineckate to form derivatives which are useful for their identification and determination. Microscopic examination and melting point determinations of the isolated complexes serve as useful means for the characterisation of many basic reineckates[1–6]. Reineckates can be quantitatively determined either gravimetrically, colorimetrically or by titration[7–16].

Although microscopic examination and melting point determinations are useful they are often insufficient to permit unequivocal identification of reineckates which possess similar micro-crystals or overlapping decomposition temperatures. The ultra-violet and visible absorption spectra are now shown to offer additional parameters for distinguishing these reineckates. The regeneration of the conjugate bases from their reineckates using ion exchange resins has also been investigated.

EXPERIMENTAL

Apparatus, reagents and solutions. Beckman Model DU Spectrophotometer; 1 cm. quartz cells; A.R. acetone; ammonium reineckate solution, approximately 2 per cent solution prepared by dissolving 2 g. of ammonium reineckate in 100 ml. cold water and filtering through a Whatman No. 42 paper; ion exchange resin Permutit De-Acidite FF. The bases used as listed in the tables were commercial products and were not further recrystallised.

Preparation of Reineckates of Mono-basic Compounds

Excess ammonium reineckate solution was added slowly with constant stirring to a solution of the base in 0·1N hydrochloric acid. After cooling to 0° the precipitate was filtered and washed with water to remove excess ammonium reineckate solution. The products were purified by recrystallisation in 60 per cent ethanol at 60° (higher temperatures cause decomposition of some reineckates). Usually two recrystallisations gave compounds sufficiently pure for physico-chemical characterisation.

Preparation of Di-reineckates of Dibasic Compounds

About 100 mg. of the dibasic compound or its salt was dissolved in 20 ml. of 0·1N hydrochloric acid. Excess ammonium reineckate solution

was added and the precipitate formed was filtered and dried over phosphorus pentoxide. The di-reineckates thus obtained were sufficiently pure for chromium determination. Repeated recrystallisation from 60 per cent ethanol gave the mono-derivatives of most of the substances studied with the exception of quinine, doxylamine and mepyramine di-reineckates.

TABLE I

PHYSICO-CHEMICAL CHARACTERISATION OF AMINE-REINECKATES

Base	Mol. composition of reineckate	Decomposition temperature °C.	Chromium analyses		Molecular weight		E (1 g.mol./l., 1 cm.)	Solubility in water g./100 ml.	
			Calc. per cent	Found per cent	Calc.	Found		27° C.	5° C.
Opium alkaloids									
Morphine	$C_{21}H_{16}CrN_7O_8S_4$	204–208	8·61	8·65	604	599	105·8	0·008	0·008
Codeine	$C_{22}H_{28}CrN_7O_8S_4$	189–193	8·41	8·47	618	618	106·4	0·021	0·011
Thebaine	$C_{23}H_{18}CrN_7O_8S_4$	196–200	8·25	8·27	630	631	106·6	0·002	0·003
Narcotine	$C_{26}H_{30}N_7O_7S_4$	150–153	7·10	7·18	732	745	104·9	0·010	0·010
Papavarine	$C_{24}H_{18}N_7O_7S_4$	207–211	7·91	8·05	—	—	110·0	0·0	0·0
Synthetic narcotics									
Ketobemidone	$C_{19}H_{26}CrN_7O_8S_4$	173–176	9·18	9·12	566	564	106·1	0·007	0·005
Pethidine	$C_{19}H_{28}CrN_7O_8S_4$	136–138	9·18	9·26	566	565	106·3	0·019	0·011
Alphaprodine	$C_{20}H_{28}CrN_7O_8S_4$	172–174	8·96	9·01	578	583	107·4	0·008	0·006
Pipidone	$C_{28}H_{38}CrN_7OS_4$	164–166	7·78	7·82	668	665	106·2	0·021	0·006
Methadone	$C_{25}H_{34}CrN_7OS_4$	160–164	8·28	8·36	628	630	106·7	0·019	0·011
Phenadoxone	$C_{27}H_{36}CrN_7O_8S_4$	162–165	7·76	7·79	670	675	107·1	0·009	0·010
Racemorphan	$C_{21}H_{30}CrN_7OS_4$	165–167	9·02	8·97	576	581	107·3	0·013	0·007
Metopon	$C_{29}H_{28}CrN_7O_8S_4$	246–253	8·42	8·32	618	628	106·9	0·035	0·012
Diacetylmorphine	$C_{25}H_{30}CrN_7S_4O_5$	180–185	7·55	7·58	688	682	105·7	0·020	0·015
Sulphonamides									
Sulphacetamide	$C_{12}H_{17}CrN_8O_8S_5$	134–137	9·76	9·67	—	—	—	0·380	0·360
Sulphathiazole	$C_{13}H_{16}CrN_8O_8S_6$	172–175	9·08	9·22	574	573	106·4	0·076	0·028
Sulphadiazine	$C_{14}H_{17}CrN_{10}O_8S_5$	192–194	9·15	9·06	569	567	106·1	0·045	0·022
Sulphapyridine	$C_{15}H_{18}CrN_9O_8S_5$	177–180	9·16	9·27	568	568	106·5	0·019	0·019
Sulphamerazine	$C_{15}H_{19}CrN_{10}O_8S_5$	189–191	8·94	8·81	583	580	106·1	0·053	0·028
Sulphamethazine	$C_{16}H_{21}CrN_{10}O_8S_5$	144–147	8·71	8·52	597	595	106·1	0·032	0·022
Antihistamines									
Diphenhydramine	$C_{21}H_{25}CrN_7OS_4$	178–180	9·07	9·12	574	575	106·8	0·016	0·04
Promethazine	$C_{21}H_{17}CrN_8S_5$	155–157	8·62	8·51	603	604	106·8	0·001	0·0
Pecazine	$C_{23}H_{19}CrN_8S_5$	188–190	8·29	8·27	629	626	106·2	0·0	0·0
Antazoline	$C_{21}H_{23}CrN_9S_4$	159	8·91	8·85	584	581	106·1	0·032	0·0
Phenindamine	$C_{25}H_{26}CrN_7S_4$	148–150	8·96	8·80	580	574	105·4	0·008	0·005
Doxylamine	$C_{21}H_{29}CrN_8S_4O$	145	8·84	8·84	589	587	106·3	0·004	0·0
Doxylamine	$C_{35}H_{36}Cr_2N_{14}OS_8$*	153–155	11·45	11·45	—	—	—	0·008	0·0
Mepyramine	$C_{21}H_{29}CrN_9OS_4$	142–143	8·62	8·59	603	604	106·6	0·003	0·0
Mepyramine	$C_{35}H_{47}Cr_2N_{14}OS_8$*	135–138	11·05	11·08	—	—	—	0·004	0·0
Methapyrilene	$C_{20}H_{24}CrN_9S_5$	152–155	8·96	8·78	580	581	106·7	0·027	0·0
Methapyrilene	$C_{32}H_{38}Cr_2N_{15}S_9$*	144–147	11·55	11·55	—	—	—	0·013	0·007
Chlorothan	$C_{18}H_{15}ClCrN_8S_5$	162–165	8·46	8·44	615	614	106·3	0·009	0·008
Chlorothan	$C_{32}H_{32}ClCr_2N_{15}S_9$*	134–138	11·15	11·15	—	—	—	0·0	0·0
Chlorpheniramine	$C_{20}H_{16}ClCrN_8S_4$	130–135	8·78	8·80	593	593	106·5	0·0	0·0
Chlorpheniramine	$C_{34}H_{32}ClCr_2N_{14}S_8$*	103–107	11·4	11·30	—	—	—	0·001	0·0
Chlorcyclizine	$C_{22}H_{18}ClCrN_8S_4$	190–193	8·42	8·42	619	616	106·1	0·024	0·016
Chlorcyclizine	$C_{36}H_{35}ClCr_2N_{14}S_8$*	162–164	11·09	11·02	—	—	—	0·0	0·0
Thonzylamine	$C_{20}H_{29}CrN_{10}OS_4$	154–157	8·60	8·43	605	604	106·3	0·0	0·0
Thonzylamine	$C_{34}H_{36}Cr_2N_{16}OS_8$*	136–138	11·24	11·29	—	—	—	0·028	0·015
Antergan	$C_{21}H_{29}CrN_8S_4$	150–153	9·08	9·11	573	574	106·7	0·004	0·0
Antergan	$C_{35}H_{36}Cr_2N_{14}S_8$*	105–107	11·65	11·71	—	—	—	0·0	0·0
Others									
Quinine	$C_{24}H_{31}CrN_8O_2S_4$	216–218	8·10	8·00	643	640	106·2	0·007	0·003
Quinine	$C_{28}H_{38}Cr_2N_8O_2S_8$*	145–149	10·80	10·92	—	—	—	0·004	0·00
Cocaine	$C_{31}H_{28}CrN_7O_8S_4$	159–162	8·35	8·39	622	624	106·8	0·009	0·005
Phenazone	$C_{15}H_{19}CrN_8OS_4$	155–157	10·25	10·17	—	—	—	0·034	0·007
Amphetamine	$C_{13}H_{20}CrN_7S_4$	132	11·46	11·43	454	452	106·2	0·120	0·117
Caffeine	$C_{12}H_{17}CrN_{10}O_8S_4$	137–138	10·10	9·84	—	—	—	0·625	0·312
Theobromine	$C_{11}H_{15}CrN_{10}O_8S_4$	165–167	10·45	10·23	—	—	—	0·990	0·445
Aniline	$C_{10}H_{14}CrN_7S_4$	195–197	12·65	12·68	412	411	106·2	—	—
Pyridine	$C_9H_{12}CrN_7S_4$	198–202	13·05	13·17	398	395	105·5	0·037	0·018

* Di-reineckates.

Preparation of Mono-reineckates of Dibasic Compounds

Procedure 1. Repeated recrystallisation of di-reineckates in 60 per cent aqueous ethanol. The di-reineckates were recrystallised thrice from 60 per cent aqueous ethanol at a temperature not exceeding 60°. Under these conditions most of the di-reineckates gave the pure mono-derivatives with the exceptions already stated.

Procedure 2. Formation of the reineckates at 70°. An aqueous solution containing a salt of a dibasic compound was heated to 70°. Ammonium reineckate solution was added with stirring. The solution was cooled and the precipitate filtered. Chromium analyses on the products indicated that all the dibasic substances gave the mono-reineckates with the exception of quinine, doxylamine and mepyramine.

Procedure 3. Formation in alkaline media. An aqueous solution of a salt of a dibasic compound was added to a slightly ammoniacal solution of ammonium reineckate. The mono-reineckate was filtered and dried over phosphorus pentoxide. Chromium analyses showed that compounds prepared by this procedure were always the mono-reineckates. This procedure is a general one for the direct preparation of the mono-derivatives of dibasic compounds. Prolonged standing of the ammoniacal solution is to be avoided as occasionally a purplish contaminating precipitate was also formed. However, the pure mono-reineckates can still be obtained by recrystallisation of the contaminated reineckates from 60 per cent ethanol.

Determination of Molecular Weights

About 10 to 15 mg. of the recrystallised reineckate complex was dissolved in 5 ml. acetone in a 5 ml. volumetric flask. The optical density was measured at 525 mμ and the molecular weight of the reineckate calculated from the following formula:

$$M = w/A \times \epsilon/v \times 1{,}000 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where M = molecular weight of the reineckate in g., w = mg. of the reineckate used, A = observed optical density, ϵ = gram-molecular extinction coefficient of the reineckate ($\epsilon_{525 m\mu} = 106{\cdot}5$), and v = volume of acetone used. The molecular weights obtained by this procedure for certain compounds are shown in Table I, column 5.

Determination of the Solubilities of the Reineckates in Water

The reineckate complex was added to water in a 25 ml. volumetric flask until no more went into solution. At this stage more reineckate was added to ensure that the solution was saturated. This solution was allowed to stand in a bath of the required temperature for one hour with constant shaking. The solution was filtered through a Whatman No. 42 filter paper and the optical density of the filtrate measured at 525 mμ. The solubilities of the reineckates were calculated from the following formulae:

$$w = A/\epsilon \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$w = A/2\epsilon \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where w = mg. of reineckate dissolved in 25 ml. of water, A = observed optical density, v = volume of water used (25 ml.) and M = molecular weight of the reineckate, and ϵ = gram-molecular extinction coefficient of ammonium reineckate in water ($\epsilon_{525m\mu} = 108{\cdot}5$). Equation (2) is used for the calculation of the solubilities of mono-reineckates and equation (3) for di-reineckates.

The solubilities of the various reineckates studied are shown in Table I, column 7.

Quantitative Determination of Organic Bases

In a 50 ml. beaker about 10 mg. of the base or its salts was dissolved in 5 ml. of 0·1N hydrochloric acid. The beaker was placed in an ice bath

TABLE II

ASSAY OF DRUGS* BY AMMONIUM REINECKATE

Compounds	Mol. composition of compounds	Mol. wt.	Amount used mg.	Optical density A at 525 mμ	Amount calc. mg.
Synthetic Narcotics					
Levomethorphan hydrobromide ..	$C_{18}H_{25}NO{\cdot}HBr$	352·32	13·8	0·416	13·75
Dextromethorphan hydrobromide ..	$C_{18}H_{25}NO{\cdot}HBr$	352·32	9·25	0·280	9·25
Levomethadone hydrochloride ..	$C_{21}H_{27}NO{\cdot}HCl$	345·90	24·0	0·741	24·15
Dextromethadone hydrochloride ..	$C_{21}H_{27}NO{\cdot}HCl$	345·90	11·4	0·345	11·2
Pipadone	$C_{24}H_{31}NO{\cdot}HCl$	386·00	13·2	0·363	13·2
Ketobemidone	$C_{15}H_{21}NO_2{\cdot}HCl$	283·79	13·8	0·518	13·8
Acetoxyketobemidone	$C_{17}H_{23}NO_3{\cdot}HCl$	325·84	19·9	0·650	19·8
DL-Methadone hydrochloride ..	$C_{21}H_{27}NO{\cdot}HCl$	349·90	22·4	0·700	22·9
Antihistamines					
Promethazine hydrochloride ..	$C_{17}H_{20}N_2S{\cdot}HCl$	320·89	15·8	0·531	16·0
Diphenhydramine hydrochloride ..	$C_{17}H_{21}NO{\cdot}HCl$	291·83	15·0	0·556	15·2
Antazoline hydrochloride	$C_{17}H_{19}N_3{\cdot}HCl$	301·83	17·05	0·607	17·15
**Chlorothan citrate	$C_{14}H_{19}ClN_2S{\cdot}C_6H_8O_7$	487·98	17·7	0·786	17·9
**Chloropheniramine maleate ..	$C_{16}H_{19}ClN_2{\cdot}C_4H_4O_4$	390·88	10·0	0·557	10·2
**Chlorcyclizine hydrochloride ..	$C_{18}H_{21}ClN_2{\cdot}HCl$	337·30	16·1	1·030	16·3
**Doxylamine succinate	$C_{17}H_{22}N_2O{\cdot}C_4H_6O_4$	388·47	12·65	0·727	12·9
**Methapyrilene hydrochloride ..	$C_{14}H_{19}N_3S{\cdot}HCl$	297·86	14·0	1·020	14·2
**Thonzylamine hydrochloride ..	$C_{16}H_{22}N_4O{\cdot}HCl$	322·85	12·1	0·804	12·2
**Antergan	$C_{17}H_{22}N_2{\cdot}HCl$	290·81	12·3	0·925	12·6
**Mepyramine maleate	$C_{17}H_{23}N_3O{\cdot}C_4H_4O_4$	401·47	9·2	0·492	9·3
Sulphonamides					
Sulphamerazine	$C_{11}H_{12}N_4O_2S$	264·32	10·0	0·413	10·25
Sulphathiazole	$C_9H_9N_3O_2S$	255·33	10·0	0·430	10·25
Sulphapyridine	$C_{11}H_{11}N_3O_2S$	249·30	15·0	0·650	15·2
Sulphadiazine	$C_{10}H_{10}N_4O_2S$	250·29	15·0	0·645	15·1
Others					
Antipyrine	$C_{11}H_{12}N_2O$	188·22	15·9	0·863	15·25
2-Azobicyclo (3,3,1) nonane hydrochloride	$C_8H_{15}N{\cdot}HCl$	161·67	12·55	0·810	12·3

* Drugs used are commercial products, not recrystallised.

** Substances which form di-reineckates.

and 10 ml. of ammonium reineckate solution added. The contents of the beaker was cooled to 0°. The precipitate was filtered through a sintered glass filtering funnel and washed with 1 ml. portions of ice cold water until the wash liquid was colourless. To remove excess water from the precipitate suction was continued. The precipitate was dissolved in acetone and transferred to a 10 ml. volumetric flask and diluted with acetone to exactly 10 ml. volume. The absorption was measured at 525 mμ. The amounts of bases or their salts present can be calculated

by using equation (4) for substances which form mono-reineckates, or equation (5) for compounds which form di-reineckates.

$$w = A/106{\cdot}5 \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad (4)$$

$$w = A/213{\cdot}0 \times v/1{,}000 \times M \quad .. \quad .. \quad .. \quad .. \quad (5)$$

w = weight of base or salt in mg., A = observed optical density, v = volume of acetone used, M = molecular weight of base or salt. Tables II and III show some recovery experiments using this procedure.

TABLE III

ASSAY OF PURE ALKALOIDS* AND MANUFACTURED OPIATES* USING AMMONIUM REINECKATE

Alkaloid	Mol. composition of alkaloid	Mol. wt.	Amount used mg.	Optical density A at 525 mμ	Amount calc. mg.
Morphine	$C_{17}H_{19}NO_3{\cdot}H_2O$	303·35	5	0·180	5·1
			10	0·356	10·1
Morphine hydrochloride	$C_{17}H_{19}NO_3{\cdot}HCl{\cdot}3H_2O$	375·84	10	0·286	10·1
Codeine	$C_{18}H_{21}NO_3$	299·36	5	0·176	4·95
			10	0·346	9·8
Codeine phosphate	$C_{18}H_{21}NO_3{\cdot}H_3PO_4{\cdot}1\frac{1}{2}H_2O$	424·38	17·9	0·455	18·1
Thebaine	$C_{19}H_{21}NO_3$	311·37	5	0·170	5·0
			10	0·342	10·0
Thebaine hydrochloride	$C_{19}H_{21}NO_3{\cdot}HCl{\cdot}H_2O$	365·85	10	0·287	9·9
Narcotine	$C_{22}H_{23}NO_7$	413·41	5	0·132	5·1
			10	0·258	10·0
Narcotine hydrochloride	$C_{22}H_{23}NO_7{\cdot}HCl{\cdot}\frac{1}{2}H_2O$	458·88	10	0·230	9·9
Dihydromorphine	$C_{17}H_{21}NO_3{\cdot}H_2O$	305·37	17·7	0·630	17·9
Dihydromorphine hydrochloride	$C_{17}H_{21}NO_3{\cdot}HCl$	323·80	21·8	0·707	21·5
Dihydrocodeinone hydrochloride	$C_{18}H_{21}NO_3{\cdot}HCl$	335·82	21·0	0·650	20·5
Benzylmorphine hydrochloride ..	$C_{24}H_{25}NO_3{\cdot}HCl$	411·91	22·2	0·562	21·8
Dihydrocodeinone	$C_{18}H_{21}NO_3$	299·37	23·0	0·827	23·5
Morphine-N-oxide	$C_{17}H_{19}NO_4$	301·33	15·9	0·563	15·9

* Drugs used are commercial products not recrystallised.

Ultra-violet Absorption Spectra

About 10 mg. of the reineckate salt was dissolved in 100 ml. of 95 per cent ethanol and 10 ml. of this solution was further diluted to 50 ml. with 95 per cent ethanol to give a solution containing about 2 mg. of reineckate per 100 ml. of ethanol. The spectra obtained are shown in Figures 3 and 4, a–d.

Regeneration of the Conjugate Bases from their Reineckates

The anion exchange column (1 cm. diameter) was filled with Permutit De-Acidite FF resins to a drain height of about 10 cm. The resin was converted to the OH form by treatment with 50 ml. 0·5N sodium hydroxide. The column was then washed with water until the effluent has a pH of 7.

About 10 to 20 mg. of the reineckate in 50 ml. of acetone was passed through the column until the eluate gave a negative test with Mayer's reagent. This acetone eluate was evaporated to dryness on a steam bath and the residue which is the conjugate base can be subjected to further confirmatory tests if required.

The column was re-activated by washing first with distilled water followed with 50 ml. of 0·5N sodium hydroxide solution. Results obtained with this procedure were very satisfactory.

Results and Discussions

The Formation and Recrystallisation of Reineckates

The formation of the reineckates depends on the pKb values of the bases and the pH of the reaction media[17]. This view was later shared by Poethke and others[4]. It is believed that the reaction proceeds via the protonation of the base B to the conjugate acid BH^+ which then reacts with the reineckate ion to form the complex thus:

$$BH^+ + [Cr(NH_3)_2(SCN)_4]^- \rightarrow BH\,[Cr(NH_3)_2(SCN)_4] \quad .. \quad (6)$$

The equilibrium between the conjugate acid BH^+ and water is

$$BH^+ + H_2O \rightleftharpoons H_3O^+ + B \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

This equilibrium is governed by the strengths of the bases and the pH values of the reaction media. The relation between these variables may be expressed by the following equation:

$$\log[B]/[BH^+] = pH + pKb - 14 \quad .. \quad .. \quad .. \quad .. \quad (8)$$

Since dibasic substances have two pKb values equation (8) above can thus be used to explain the formation of mono- and di-reineckates in different pH media. The mechanism of the reaction between ammonium reineckate and organic bases has been studied in detail and will form the subject of another paper.

The formation of both the mono- and di-reineckates in acid media presented no difficulties. Recrystallisation of the mono-reineckates from 60 per cent ethanol gave the pure products. However, recrystallisation of all the di-reineckates studied with the exception of quinine, doxylamine and mepyramine yielded the mono-derivatives under these conditions. Many of the di-reineckates are unstable to heat. When ammonium reineckate is added to acidic aqueous solution containing dibasic compounds at 70° the mono-reineckates are usually obtained with the exception of the three compounds mentioned earlier.

Spectral Characteristics of the Absorption Spectra of the Reineckates, their Uses and Limitations

The spectral curves of several reineckates studied in acetone solution between 350–600 mμ were found to be similar to that of ammonium reineckate itself (Fig. 1) with the exception of papaverine[12] and cotarnine reineckates. This phenomena has been observed by other workers[3,13,16]. The absorption of the reineckates is attributed exclusively to the reineckate moiety of the molecule and is independent, with the exceptions stated, of the conjugate base. Examination of the ammonium reineckate curve shows two maxima at 395 mμ and 525 mμ. The average values of $\epsilon_{525 m\mu}$ for reineckates listed in column 6 of Table I are 106·5 and these are in excellent agreement with the observed values for ammonium reineckate itself.

From the spectral relationships stated, the following formula can be found when a cell of 1 cm. path length is used:—

$$w/M = A/\epsilon \times v/1{,}000 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

This equation can be used for the determination of the molecular weights and solubilities of the reineckates and also for the quantitative determination of certain organic bases without the use of calibration curves.

The accuracy of the spectrophotometric method of molecular weight determination depends mainly on the accuracy of the weighing process,

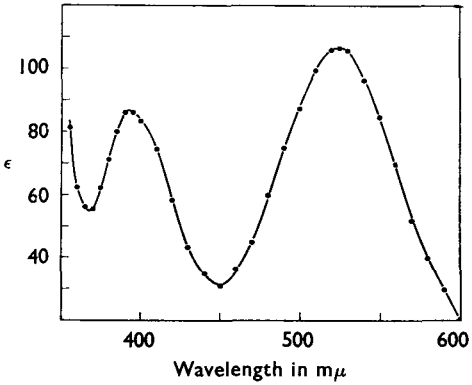

FIG. 1. Ultra-violet absorption curve of ammonium reineckate monohydrate in acetone.

the exact determination of the optical density of the solution at the chosen wavelength and the purity of the reineckates studied. Normally about 15 mg. of the reineckate, representing about 7 mg. of the conjugate base is used and a small error in weighing or the reading of the absorbance may lead to an appreciable error in the value of the molecular weight. If

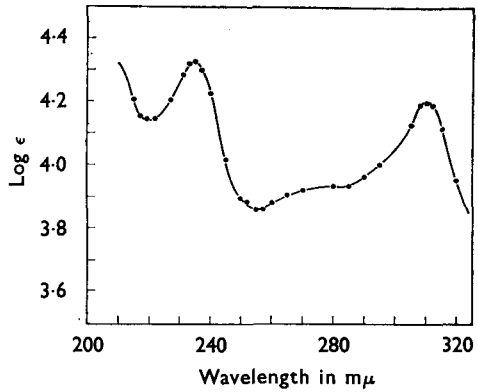

FIG. 2. Ultra-violet absorption curve of ammonium reineckate monohydrate in ethanol.

larger quantities are used better results are obtained. Table I, column 5, lists the molecular weights of the reineckates obtained by this method. The molecular weights of the free bases can be obtained by subtracting 319, which is the molecular weight of the reinecke acid, from these values.

The solubilities of the various reineckates are shown in Table I, column 7. This method of determination of the solubilities of the reineckates is superior to existing methods in that it is simple and measures directly

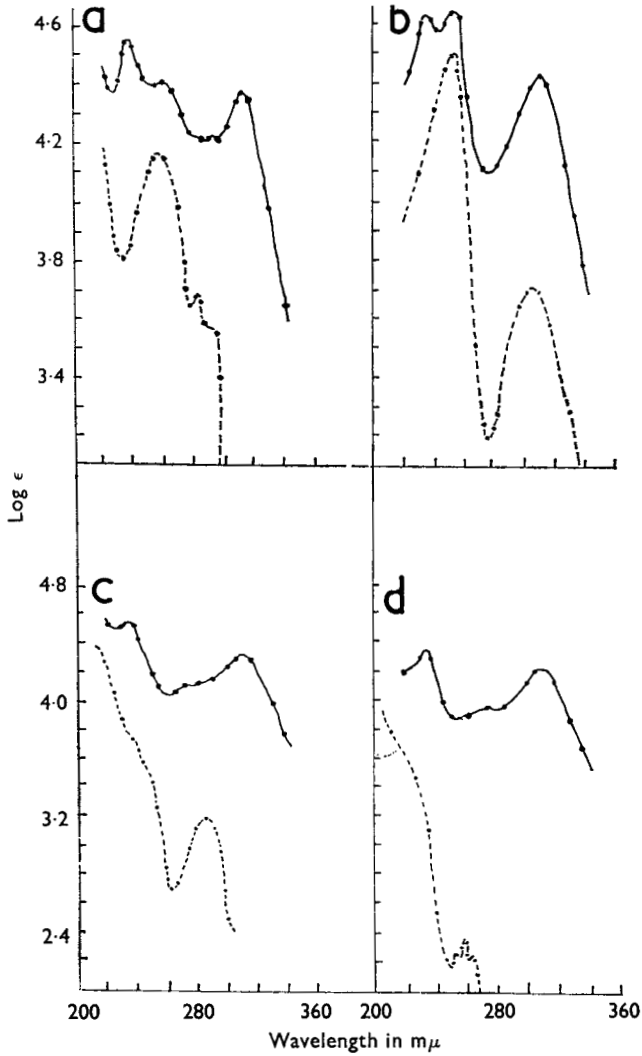

FIG. 3. Ultra-violet absorption curves for: a, strychnine reineckate (·——·) and base (· - - - ·); b, pecazine reineckate (·——·) and hydrochloride (· - - - ·); c, morphine reineckate (·——·) and base (· - - - ·); d, pethidine reineckate (·——·) and base (· - - - ·). Solvent 95 per cent ethanol.

the amounts that have gone into solution. An examination of these data reveals that in general the solubilities of the reineckates decrease with temperature. The solubilities of the reineckates appear to be a function of the pKb values of the conjugate bases and are not dependent

on whether they are derived from primary, secondary or tertiary amines as reported[19].

The data in Tables II and III illustrate that the formulae (4) and (5) can be used for the quantitative determination of many of the bases without the use of standard calibration curves. The recoveries are generally good as shown in the tables. These formulae are only applicable to substances whose reineckates are not too soluble in water and are obviously not applicable for the determination of weak bases such as caffeine, theobromine and sulphacetamide.

The ultra-violet absorption spectra of ammonium reineckate is shown in Figure 2. This curve has a maximum at 235 mμ and another at 310 mμ together with an almost flat portion of the curve between its minima at 255 mμ and 300 mμ. All the spectra in Figures 3 and 4, a–d, shown, with the exception of morphine and pethidine, are characteristic of the bases they represent in that they all possess maxima at the wavelengths

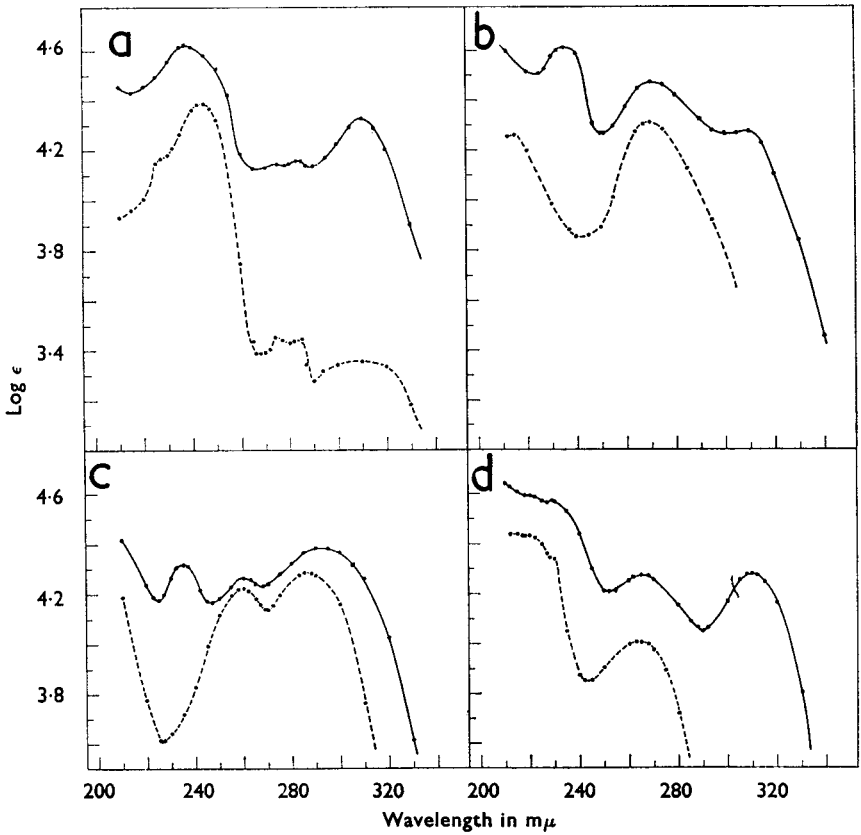

FIG. 4. Ultra-violet absorption curves for: a, thonzylamine monoreineckate (·——·), and hydrochloride (· - - - ·); b, sulphamerazine reineckate (·——·) and sulphamerazine (· - - - ·); c, sulphathiazole reineckate (·——·) and sulphathiazole (· - - - ·); d, phenindamine reineckate (·——·) and tartrate (· - - - ·). Solvent 95 per cent ethanol.

corresponding to the maxima of the spectra of the conjugate bases. Besides morphine and pethidine, the spectra of codeine, ketobemidone and methadone reineckates were also found not to have any maxima at the wavelengths corresponding to the maxima of the spectra of these substances.

Recovery of the Amines from their Reineckates

The regeneration of the conjugate bases from their reineckate derivatives using Permutit De-Acidite FF ion exchange resins presented no difficulties. The reaction between the resin and the reineckates can be represented by the following equation:

$$[RCH{\cdot}N(R^1)_3{}^+\,OH^-] + BH\,[Cr(NH_3)_2(SCN)_4] \rightarrow \{RCH{\cdot}N(R^1)_3{}^+\,[Cr(NH_3)_2(SCN)_4]^-\} + B + H_2O \quad .. \quad (10)$$

It was found that the free base B set free in accordance with equation (10) remained in the acetone solution and that very little acetone was required to elute from the resin bed any material which had precipitated during the ion exchange reaction. Evaporation of the acetone yielded substances pure enough for further confirmatory tests.

This method of regeneration of the bases is superior in simplicity of operation to the Kapfhammer method[18], that is by treating an acetone solution of the reineckate with silver sulphate and then with barium chloride. The only limitation to the use of the present procedure for the liberation of the conjugate bases is that the De-Acidite FF resin is a strong anion exchanger and holds back amphoteric bases such as morphine and certain sulphonamides on the column. However, these substances can be eluted from the column by using 10 per cent acetic acid solution. The use of this procedure for the isolation of alkaloids from plant materials has already been reported[20].

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