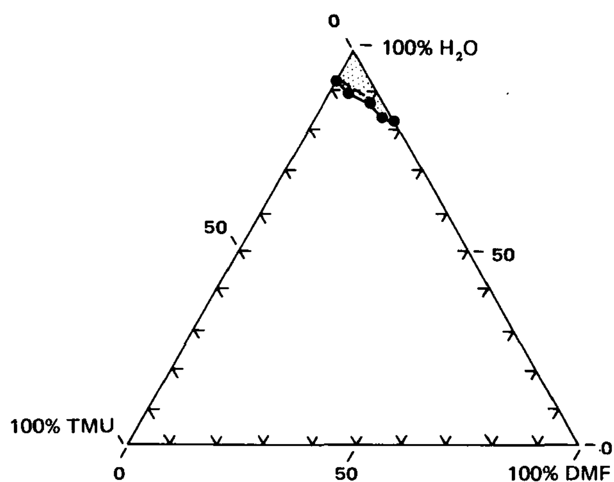

Figure 10—*Ternary diagram for aqueous dimethylformamide (DMF)-tetramethylurea (TMU) solutions containing 0.9% sodium chloride at 37°.*

for this compound. All other solvents have been reported to offer very little protection to red blood cells from hemolysis at all concentrations (1, 4–6). Therefore, the convex deviation from the theoretical tie-line can be explained by the fact that polyethylene glycol 400 contributed to the tonicity of the ternary solvent by virtue of its intrinsic property of incomplete penetration into the red blood cell.

The components of ternary solvent systems not containing polyethylene glycol 400 do contribute to the tonicity of the system but to a lesser degree than the polyethylene glycol 400 systems. Hemolytic isotonic coefficients (i values) were reported previously for sodium chloride in all solvents used in this study (1, 4–6). These i values for sodium chloride in aqueous solutions containing approximately one-half the critical hemolytic concentrations of the solvents were 1.71 for 4% tetramethylurea (6), 1.90 for 10% dimethylformamide (5), 1.95 for 15% propylene glycol (1), and 2.10 for 20% dimethyl sulfoxide (4).

When the isotonic coefficients are used as an indicator of the individual solvent's contribution to the tonicity of extracellular solutions, tetramethylurea would have the least contribution and dimethyl sulfoxide the most. Therefore, according to these hemolytic i values, the aqueous tetramethylurea–dimethylformamide system (average i value 1.80) should contribute very little, if at all, to the extracellular tonicity and should display little deviation from the theoretical tie-line. The experimental data (Fig. 10) are in agreement with this explanation.

The experimental data for the other systems also concur with this explanation, since the rank order of the average sodium chloride i values of the two nonaqueous components in a particular ternary system was approximately the same as the rank order of the degree of deviation of the experimental curves from their theoretical tie-lines. The rank order for the amount of deviation from the theoretical tie-line was propylene glycol–dimethyl sulfoxide (i = 2.03) > dimethylformamide–dimethyl sulfoxide (i = 2.00) > dimethylformamide–propylene glycol (i = 1.93) = tetramethylurea–dimethyl sulfoxide (i = 1.92) > tetramethylurea–propylene glycol (i = 1.83) > tetramethylurea–dimethylformamide (i = 1.80).

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Analysis of *Duboisia myoporoides* R. Br. and *Duboisia leichhardtii* F. Muell.

W. J. GRIFFIN [x], H. P. BRAND, and J. G. DARE

Abstract ☐ *Duboisia* samples were analyzed for scopolamine and hyoscyamine using GLC, by a slope ratio method, and by methods employing homatropine, tetraphenylethylene, and phenylacetyltropine as internal standards. Limits of precision were determined by the inverse prediction method. Phenylacetyltropine was the preferred internal standard. The alkaloids were silanized with either hexamethyldisilazane or N,O-bis(trimethylsilyl)acetamide to prevent dehydration to the apo forms. Samples from a commercial bale of *Duboisia myoporoides* were assayed. The alkaloid content varied considerably, depending on the sample position within the bale.

Keyphrases ☐ *Duboisia myoporoides* and *Duboisia leichhardtii*—GLC analysis for scopolamine and hyoscyamine, slope ratio method ☐ Scopolamine and hyoscyamine—GLC determination in *Duboisia* samples, slope ratio method ☐ Phenylacetyltropine—internal standard in GLC analysis of scopolamine and hyoscyamine in *Duboisia* samples

Two species of *Duboisia, Duboisia leichhardtii* and *Duboisia myoporoides,* are commercial sources of the mydriatic tropane alkaloid scopolamine, 1000 tons being grown annually. They are cultivated together with their hybrids, and it was essential that an accurate analytical method for the major alkaloids, scopolamine and hyoscyamine, be established for cultivation trials. The assay is complicated by the wide spectrum of tropane alkaloids the genus offers (Table I). Coulson and Griffin (1) found that aerial parts of

D. myoporoides contain 12 different tropane alkaloids. The complex nature is complemented by the reported presence of the pyridine alkaloids, nicotine and anabasine (2).

Various analytical procedures for the assessment of scopolamine and hyoscyamine have been published. Solomon *et al.* (3) were the first to apply GLC to the quantitation of scopolamine and atropine in plant powders. A standard linear regression line was established of quantity *versus* peak area. For the nine results quoted, the amounts estimated differed from the actual amounts by as much as 27.5%, although five differed by less than 4%. On-column decomposition of the alkaloids to their apo forms also occurred.

An assay procedure was described for unit dose quantities of scopolamine, using homatropine as an internal standard (4, 5). The amounts estimated differed from the stated actual amount by as much as 14%. The use of tetraphenylethylene as an internal standard was described (6).

This paper reports the use of phenylacetyltropine as an internal standard and gives a critical appraisal of various assays. Where estimations from a known regression were made, predicted inverse tolerance limits were obtained according to Williams (7).

Table I—GLC Retention Times of *Duboisia* Alkaloids[a]

Alkaloids	A[b]	B[c]
Tetraphenylethylene[d]	1.27	1.30
Scopolamine	1.0	1.0
Hyoscyamine	0.73	0.69
Norhyoscyamine	0.74	0.69
Homatropine[d]	0.53	0.47
Meteloidine[e]	0.37	0.31
Phenylacetyltropine[d]	0.36	0.27
Valeroidine	0.18	0.13
Tigloidine	0.15	0.10
Valtropine	0.12	0.07
Butropine	0.09	0.05
Anabasine	0.09	0.05
Nicotine	0.07	0.03
Acetyltropine	0.06	0.03

[a] Actual retention time = (A) × 15 min and (B) × 32.8 min. All alkaloids were treated with hexamethyldisilazane. [b] Column temperature was 233°, column pressure was 15 psi, and flow rate was 60 ml/min. [c] Column temperature was 194°, column pressure was 28 psi, and flow rate was 190 ml/min. [d] Internal standards. [e] A tropane alkaloid not isolated from *Duboisia*.

EXPERIMENTAL[1]

Materials—The carrier gas was helium. Dual columns of Pyrex glass (150 cm × 4 mm i.d.) were packed with acid-washed, silanized Chromosorb W (80–100 mesh) containing 1.5% SE-30. The silanized support material was coated with a trace of polyethylene glycol 4000 and then with SE-30 according to the method of Street (8). The packing was held by a small plug of silanized glass wool.

All solvents were fractionally distilled, and the chemicals used were of analytical reagent grade. Plant material was dried at 50° and then milled[2] to a moderately coarse powder (BP). Hyoscyamine base[3], scopolamine hydrobromide[3], homatropine[3], hexamethyldisilazane[4], *N,O*-bis(trimethylsilyl)acetamide[5], and tetraphenylethylene[6] were obtained commercially. Phenylacetyltropine was prepared by chemical synthesis.

Slope Ratio Assay—The column temperature was 194° with an injection port heater set at 244°. The gas inlet pressure was 28 psi and gave a flow rate of 190 ml/min. The output was attenuated ×2000 to a 10-mv recorder with a chart speed of 20 mm/min. Peak areas were measured by the peak height–peak width method (9).

Solutions were prepared by dissolving scopolamine in pyridine containing 5% hexamethyldisilazane on the day before use. By forming the trimethylsilyl derivative, on-column decomposition to the apo form was avoided and symmetrical peaks with no tailing were obtained. Both the standard and unknown preparations were injected on the column in five replicates using two separate volumes, 10 and 20 μl, to simplify the statistical evaluation.

Several assays (A–C) were performed in which the exact milligrams per milliliter content of scopolamine was known in both the standard and the unknown but in which the strength of the unknown solution was not disclosed to the analyst until after the assay was completed. A commercial sample of *Duboisia* (D), previously assayed by a titrimetric method (10), was analyzed. Other samples of *Duboisia* (E–H) fortified with increasing amounts of scopolamine were assayed similarly (Table II).

A 10-g sample of coarsely powdered *D. leichhardtii*[7] leaves, mixed with 1 g of calcium hydroxide, was moistened with 10 ml of distilled water (or, in later experiments, water containing a known quantity of scopolamine hydrobromide). After 30 min to allow a thorough penetration of alkali, the mixture was exhaustively extracted by repeated maceration with cold ether (3 × 150 ml). The combined ether extracts, after concentration to 100 ml, were extracted with 0.5 *N* hydrochloric acid (2 × 100 ml), and the combined acidic extracts were basified with excess ammonium hydroxide solution (wt./ml 0.88).

The alkaloid bases were extracted with chloroform (3 × 150 ml), and the combined chloroform extracts were evaporated under reduced pressure. The residue was taken up in pyridine containing 5% hexamethyldisilazane. The solution was quantitatively transferred to a 50-ml volumetric flask, and the volume was adjusted with more solvent.

Internal Standard Assay—The column temperature was 235° with an injection port heater set at 285°. The gas inlet pressure was 15 psi and gave a flow rate of 60 ml/min. The output was attenuated ×5000 to a 10-mv integrating recorder.

Regression Line Investigation for Phenylacetyltropine and Tetraphenylethylene—Standard solutions of scopolamine hydrobromide (0.125 g/50 ml of water), phenylacetyltropine picrate (0.125 g/50 ml of chloroform), hyoscyamine (0.075 g/50 ml of chloroform), and tetraphenylethylene (0.125 g/50 ml of chloroform) were prepared. One milliliter of the phenylacetyltropine solution was added to 16 separators using a buret[8]. To every separator were added measured volumes of the scopolamine and hyoscyamine solutions (4 × 0.5, 4 × 1, 4 × 1.5, and 4 × 2 ml), so that each separator contained identical volumes of the two solutions.

The volumes of organic and aqueous phases in each separator were adjusted to 3 and 2 ml, respectively, by appropriate addition, if necessary, of chloroform and water. The extraction then was continued as follows. Approximately 1 ml of ammonium hydroxide solution (wt./ml 0.88) was added, and the two phases were shaken together. The chloroform layer was run off into a stoppered test tube, and the aqueous layer was reextracted with chloroform (2 × 2 ml). To the combined chloroform extracts was added 1 ml of the tetraphenylethylene standard solution, and the extract was evaporated under a stream of nitrogen. The residue was taken up in 1 ml of anhydrous pyridine, and 0.30 ml of hexamethyldisilazane was added. After 8 hr, 10 μl of the silanized extract was subjected to GLC analysis.

Regression Line Investigation for Homatropine—Standard solutions of scopolamine hydrobromide (0.254 g/100 ml of water), hyoscyamine (0.156 g/100 ml of chloroform), and homatropine (0.200 g/100 ml of chloroform) were prepared. Extracts were prepared as described previously with the omission of the standard solutions of phenylacetyltropine and tetraphenylethylene, the inclusion of homatropine as the internal standard, and the replacement of hexamethyldisilazane by *N,O*-bis(trimethylsilyl)acetamide as the silanizing agent.

[1] A Pye series 104 gas chromatograph fitted with dual flame-ionization detectors and a Rikadenki 10-mv integrating recorder were used.
[2] The mill was supplied by Christy and Norris Ltd., Chelmsford, U.K.
[3] Macfarlan Smith Ltd., Edinburgh, U.K.
[4] British Drug House Chemicals Ltd., Poole, U.K.
[5] Supelco, Inc., Bellefonte, Pa.
[6] Koch Light Laboratories, Colnbrook, U.K.
[7] Supplied by E. Carter and Son, cultivators of *Duboisia*.

[8] Metrohm.

Table II—Results of Slope Ratio Assays

Assay	Amount Found	Confidence Limits, $p = 0.99$ Lower	Confidence Limits, $p = 0.99$ Upper	Confidence Limits, $p = 0.99$, as Percentages	Actual Amount	Percentage Error from Actual
		Analysis of Known Solutions of Scopolamine				
A	3.5546 mg/ml	3.4945 mg/ml	3.6147 mg/ml	±1.68	3.5768 mg/ml	−0.62
B	3.6412 mg/ml	3.5953 mg/ml	3.6870 mg/ml	±1.46	3.5768 mg/ml	+1.80
C	3.5343 mg/ml	3.4705 mg/ml	3.5880 mg/ml	±1.50	3.5768 mg/ml	−0.91
Mean	3.5767 mg/ml				3.5768 mg/ml	
		Analysis of Samples of *D. leichhardtii* Leaves				
D	0.435%	0.430%	0.442%		0.44%	
E	65.747 mg	64.747 mg	66.858 mg	±1.68	65.747 mg	
F[a]	76.378 mg	73.180 mg	79.570 mg	±4.17	76.336 mg	+0.05
G[b]	82.032 mg	79.500 mg	84.500 mg	±3.00	81.181 mg	+1.04
H[c]	83.103 mg	80.210 mg	85.990 mg	±3.47	84.849 mg	+2.05

[a] E + 10.589 mg of scopolamine. [b] E + 15.434 mg of scopolamine. [c] E + 19.102 mg of scopolamine.

Table III—Regression Equations for Internal Standard Assays[a]

Internal Standard	Scopolamine	Hyoscyamine
Phenylacetyltropine (0.0025 g picrate)	$y = 0.817529x - 0.153925$	$y = 0.80385x - 0.130925$
Tetraphenylethylene (0.0025 g)	$y = 0.267702x - 0.0722498$	$y = 0.29095x - 0.082875$
Homatropine (0.0020 g)	$y = 0.834662x - 0.057589$	$y = 0.71885x - 0.026988$

[a] x = weight of scopolamine or hyoscyamine expressed in milligrams; y = ratio of integrated areas of scopolamine or hyoscyamine *versus* internal standard.

Extraction and Assay—A 10-g sample of coarsely powdered *D. leichhardtii* leaves, mixed with 1 g of calcium hydroxide, was moistened with 10 ml of distilled water. After 30 min to allow a thorough penetration of alkali, the mixture was exhaustively extracted by repeated maceration with cold ether (3 × 150 ml). On evaporation, the combined ether extracts gave a residue which was taken up in chloroform and the volume was adjusted to 25 ml. A suitable quantity (1, 2, or 4 ml) was pipetted into a separator, and 1 ml of either homatropine or phenylacetyltropine solution was added. The volume was adjusted to 5 ml if necessary with chloroform.

The chloroform extract was shaken with 0.5 *N* hydrochloric acid (1 × 5 and 1 × 3 ml), and 1 ml of ammonium hydroxide solution (wt./ml 0.88) was added to the combined acidic extract, which was extracted with chloroform (1 × 5, 1 × 3, and 1 × 2 ml). Then the chloroform extracts were combined. One milliliter of tetraphenylethylene was added if required as an internal standard. The chloroform was removed under a stream of nitrogen, the residue was dissolved in 1 ml of anhydrous pyridine, and 0.3 ml of either hexamethyldisilazane or *N,O*-bis(trimethylsilyl)acetamide was added according to the internal standard employed. After 8 hr, a 10-μl sample was subjected to GLC analysis.

Phenylacetyltropine—Atropine sulfate (5 g) was refluxed with barium hydroxide (6 g) in distilled water (200 ml) for 2 hr. After cooling, excess 2 *N* sulfuric acid was added and the barium sulfate so formed was filtered off. The filtrate was extracted with ether (2 × 150 ml) to remove tropic acid. The aqueous fraction was made alkaline with excess ammonium hydroxide solution (wt./ml 0.88) and extracted with chloroform (4 × 100 ml).

The chloroform extracts were combined and gave tropine (2 g, 95% yield) after evaporation under reduced pressure. Tropine (1 g) was added to phenylacetyl chloride (20 ml), and the mixture was heated for 2 hr on a water bath. The reaction mixture was diluted with 80 ml of distilled water and made acidic with 2 *N* sulfuric acid. Phenylacetic acid was removed by ether extraction (2 × 100 ml). The aqueous residue was made alkaline with the addition of excess ammonium hydroxide solution (wt./ml 0.88) and extracted with chloroform (3 × 100 ml). Evaporation of the combined chloroform extracts gave phenylacetyltropine which, when neutralized with 0.2 *N* sulfuric acid and treated with a saturated aqueous solution of sodium picrate, gave phenylacetyltropine picrate (2.5 g, 72% yield), mp 166°.

Anal.—Calc. for $C_{16}H_{21}NO_2 \cdot C_6H_3N_3O_7$: C, 54.1; H, 4.9; N, 11.5. Found: C, 54.4; H, 5.1; N, 11.2.

Sampling from a Bale of Duboisia *Leaf*—One bale (175 × 150 × 100 cm, approximately 175 kg) of commercial *D. myoporoides* leaves[9] was subdivided into 11 vertical layers (A–K). Then each layer was divided into 16 lots, distributed horizontally in a 4 × 4 square so that the horizontal position of each lot could be identified. From these, four lots were selected at random, namely E5, F9, G2, and J12. Each lot was subdivided into 16 samples. Four samples were selected randomly from each lot and annotated such that E5/15 indicates Layer E, Lot 5, Sample 15. Each sample was analyzed with four replicates, using phenylacetyltropine as the internal standard.

RESULTS AND DISCUSSION

Slope Ratio Assay—Two injection volumes of a standard solution, *S* (10 and 20 μl), and two identical volumes of an unknown solution, *U*, are injected into the gas chromatograph. If the two regression lines of the responses to *S* and *U* intersect at the origin, then the ratio of the concentrations of the two solutions is equal to the ratio of the slopes of the regression lines. By using the appropriate experimental design and tests of significance, an estimate of confidence limits may be calculated from internal evidence in the assay. The statistical methods used were fully discussed by Finney (11).

For Assays A, B, and C, the analyst was presented with two solutions, labeled standard and unknown. The three standards were of different concentrations, but in each case the unknown was actually a sample of one solution. Thus, the analyst was unknowingly assaying the same preparation; but since the standards differed for each assay, the slope ratios in each case were different. Individual assays showed some error, varying from −0.62 to +1.8% (Table II). Each assay provided its own limits of precision, but in one instance the true concentration was just outside the limits. Confidence limits in Assays E, F, G, and H, in which varying amounts of scopolamine were added as the hydrobromide to powdered *D. leichhardtii*

[9] The bale was supplied by G. R. Davies, Hornsby, N.S.W. A voucher specimen, annotated BRI 179202, was lodged with the Queensland Herbarium.

Table IV—Predicted x Values and Tolerance Limits Determined from Regression Equations of the Internal Standard Assay Using y Values at the Mean, Upper, and Lower Parts of the Regression Line

Internal Standard	Assay for	y Value Observed	Mean of	Predicted x Value	Limits, $p = 0.95$
Phenylacetyl-tropine	Scopolamine	4.09	1	5.19	4.61–5.77 (±0.58, ±11.2%)
		4.09	4	5.19	4.85–5.50 (±0.31, ±6%)
		1.30	1	1.78	1.16–2.38 (±0.60, ±33%)
		1.30	4	1.78	1.31–2.25 (±0.47, ±25%)
		7.0	1	8.75	8.15–9.37 (±0.62, ±7.1%)
		7.0	4	8.75	8.40–9.12 (±0.37, ±4.2%)
Phenylacetyl-tropine	Hyoscyamine	3.48	1	4.5	3.97–5.02 (±0.52, ±11.2%)
		3.48	4	4.5	4.22–4.77 (±0.27, ±6.2%)
		1.1	1	1.53	0.97–2.06 (±0.53, ±35%)
		1.1	4	1.53	1.20–1.84 (±0.31, ±20%)
		6.0	1	7.63	7.10–8.18 (±0.55, ±7.3%)
		6.0	4	7.63	7.31–7.96 (±0.33, ±4.4%)
Tetraphenyl-ethylene	Scopolamine	1.085	1	4.32	4.03–4.62 (±0.30, ±6.1%)
		1.085	4	4.32	4.16–4.48 (±0.16, ±3.6%)
		0.4	1	1.76	1.45–2.06 (±0.30, ±17%)
		0.4	4	1.76	1.57–1.94 (±0.18, ±13%)
		1.8	1	7	6.68–7.30 (±0.30, ±4.4%)
		1.8	4	7	8.81–7.18 (±0.18, ±2.7%)
Tetraphenyl-ethylene	Hyoscyamine	1.01	1	3.75	3.35–4.15 (±0.40, ±10%)
		1.01	4	3.75	3.53–3.96 (±0.21, ±5.8%)
		0.4	1	1.66	1.23–2.07 (±0.41, ±24%)
		0.4	4	1.66	1.40–1.90 (±0.24, ±11%)
		1.8	1	6.47	6.04–6.91 (±0.44, ±6.8%)
		1.8	4	6.47	6.21–6.74 (±0.27, ±4.3%)
Homat-ropine	Scopolamine	0.98	1	1.25	1.03–1.46 (±0.21, ±17%)
		0.98	4	1.25	1.13–1.36 (±0.11, ±9.2%)
		0.4	1	0.55	0.32–0.76 (±0.21, ±39%)
		0.4	4	0.55	0.41–0.67 (±0.12, ±23%)
		1.6	1	1.98	1.77–2.21 (±0.23, ±11%)
		1.6	4	1.98	1.85–2.10 (±0.12, ±7.0%)
Homat-ropine	Hyoscyamine	0.87	1	1.25	1.10–1.39 (±0.14, ±11%)
		0.87	4	1.25	1.17–1.32 (±0.07, ±6.2%)
		0.30	1	0.45	0.30–0.60 (±0.15, ±33%)
		0.30	4	0.45	0.35–0.54 (±0.09, ±19%)
		1.50	1	2.12	1.97–2.28 (±0.16, ±7.3%)
		1.50	4	2.12	2.03–2.22 (±0.10, ±4.6%)

leaves, were greater than in previous assays because they included errors incurred in the additional extraction stage.

If Assay E is assumed to be exact, errors varied from +0.05 to +2.05%. Thus, the slope ratio method appears to provide a valuable and accurate quantitative assay for scopolamine.

Internal Standard Assay—Analysis of the results gave six regression equations for values of x (milligrams of scopolamine or hyoscyamine) and of y (ratio of integrated areas of alkaloid *versus* internal standard) (Table III). Silanization of the alkaloid mixture was essential to prevent dehydration to the apo derivatives. Of the internal standards employed, homatropine suffered the disadvantage that it underwent silanization with difficulty. Hexamethyldisilazane would not cause the silanization to go to completion, and a more active agent was necessary [N,O-bis(trimethylsilyl)-acetamide]. Internal standards such as homatropine and phenylacetyltropine are superior to tetraphenylethylene, since they are introduced into the assay during the extraction stage so that any losses during extraction are reflected in the confidence limits. The results demonstrate that of the three internal standards, phenylacetyltropine and tetraphenylethylene are the standards of choice.

If it is important that the confidence limits be close, for example to deduce whether two samples are different or if one is superior to the other, then replicate determinations will vastly narrow them. Such an occasion may arise in the selection of trees for cultivation trials. In the results (Table IV), x values are quoted at the mean of y and also at values of y at the lower and upper limits of the regression line. Greater accuracy can be achieved by adjusting the experiment so that the regression line in and around the mean is employed during the assay.

The assay for hyoscyamine also includes norhyoscyamine since both alkaloids have similar retention times. Norhyoscyamine has been isolated as a minor alkaloidal component of *D. myoporoides* (1) and *D. leichhardtii* (12). This does not detract from the value of the assay since samples of *Duboisia* for commercial purposes are estimated on their scopolamine content alone.

Commercial *Duboisia* consignments are obtained from both cultivated and natural sources. In the past, *D. leichhardtii* was the most cultivated of the two species. Cultivation of the genus was hampered by the poor germination of *Duboisia* seed, and most plantations were derived from naturally set seedlings. Hills *et al.* (13) found considerable variations in alkaloid type and yield in natural stands of *Duboisia*, suggesting the existence of chemical varieties. Thus, consignments of *Duboisia*, whether cultivated or naturally obtained, are unlikely to be homogeneous in alkaloid content.

Samples were selected randomly from a bale of *D. myoporoides*, which formed part of a commercial consignment obtained from natural sources. There was a threefold variation in the scopola-

Table V—Analysis of Samples of *D. myoporoides* Leaves Obtained from a Commercial Bale

	Percentage Scopolamine			Percentage Hyoscyamine		
		Confidence Limits, $p = 0.95$			Confidence Limits, $p = 0.95$	
Sample	Amount Found	Upper	Lower	Amount Found	Upper	Lower
E5/5	0.209	0.223	0.194	0.244	0.257	0.231
E5/15	0.487	0.507	0.468	0.213	0.225	0.201
E5/10	0.415	0.432	0.398	0.163	0.175	0.151
E5/13	0.552	0.575	0.531	0.121	0.134	0.109
F9/5	0.267	0.280	0.254	0.239	0.251	0.227
F9/15	0.351	0.367	0.333	0.169	0.180	0.157
F9/12	0.223	0.236	0.210	0.103	0.115	0.090
F9/3	0.256	0.269	0.243	0.165	0.176	0.153
G2/6	0.283	0.300	0.265	0.093	0.110	0.075
G2/16	0.414	0.431	0.398	0.078	0.091	0.065
G2/8	0.218	0.231	0.205	0.067	0.080	0.054
G2/11	0.372	0.387	0.357	0.104	0.116	0.092
J12/5	0.573	0.596	0.551	0.073	0.084	0.066
J12/3	0.586	0.611	0.564	0.088	0.100	0.075
J12/10	0.569	0.592	0.547	0.086	0.099	0.073
J12/14	0.587	0.611	0.565	0.220	0.208	0.232

mine content, depending on sample position within the bale (Table V). This finding demonstrates the difficulty in obtaining a representative sample from a consignment.

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Solubilization and Stabilization of the Cytotoxic Agent Coralyne

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Abstract □ Kinetic studies were carried out on the ring opening of the quaternary nitrogen cation, coralynium ion (I), to yield 6′-acetylpapaverine (III), on the cyclization of III to yield I, and on a photochemical reaction undergone by I in aqueous solutions exposed to visible light. From the results, it was concluded that: (*a*) I and III are in facile equilibrium in aqueous solution but appreciable amounts of III do not exist in dilute solutions with pH values below 10; (*b*) the photochemical reaction of I in water (presumably a photohydration) can be reversed by lyophilization, by heating, and by increasing the pH of solutions to values greater than 12; (*c*) the photochemical reaction of I can be inhibited by protecting the aqueous solutions from visible light, and the rate in the presence of light can be reduced by increasing the concentration of I in the solution; and (*d*) although the chloride and sulfoacetate salts of I react identically and have similar solubilities in water, it is possible to prepare more concentrated and, hence, more stable solutions of the sulfoacetate salt by including sodium hydroxide in the solvent. The solubility of coralyne chloride remains about the same in dilute sodium hydroxide as in water.

Keyphrases □ Coralyne salts—photohydration, ring cleavage, pH dependence of solubility, stabilization, formation of 6′-acetylpapaverine □ Cytotoxic agents—coralyne salts, solubilization and stabilization, photohydration, pH dependence, formation of 6′-acetylpapaverine □ 6′-Acetylpapaverine—kinetics of formation from coralynium ion, cyclization □ Solubility—coralyne chloride and sulfoacetate, pH dependence

Coralyne chloride (I*a*) is a berbinium salt possessing antileukemic activity against both the P-388 and L-1210 strains (1, 2). However, the clinical evaluation of I*a* has been hampered by the relatively high expected dose of 1-2 g[1] together with the rather low aqueous solubility of about 5 mg/ml and a report[2] that solutions of the drug stored under different conditions or for varying times exhibited altered spectral characteristics relative to freshly prepared solutions.

This study was undertaken to determine the stability of coralynium ion (I) in aqueous solution with respect to chemical transformation. Previous reports led to the expectation that the most likely degradation pathways would be a ring opening to yield 6′-acetylpapaverine (III) (3) and a covalent hydration reaction (4). Although these reactions were postulated, no quantitative information about reaction rates was reported.

An investigation into the chemical stability and solubility of coralyne sulfoacetate (I*b*), isolated as the initial product in a general synthesis of I (1), suggests that I*b* may be a better coralynium salt to use in liquid dosage forms than I*a*.

EXPERIMENTAL

Materials—Coralyne chloride[3] and coralyne sulfoacetate[4] were provided by the National Cancer Institute and used without further purification. The IR spectrum of coralyne chloride used was identical to the reported spectrum[2]. Coralyne sulfoacetate has an extra absorption band at 1720 cm^{-1} due to the carbonyl group in the anionic part. 6′-Acetylpapaverine was prepared, following the

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[4] NSC 154890, lot pH 5-113-1, mol. wt. 503.5, mp 268–274° dec. [lit. (1) 278–280° dec.].