will cause reduction of Michler's ketone to the corresponding hydrol, and under the conditions of the test a deep blue or purple color develops slowly. This color is unlike that formed by the Grignard reagent. However, when unchanged magnesium (or other metal) is noted in the sample, it is recommended that the solution be filtered subsequent to hydrolysis and prior to the addition of the glacial acetic acid-iodine solution. This alteration in procedure is desirable when one removes a sample from a highly viscous or solid reaction product, such as that formed in the preparation of acids. In such cases any unchanged magnesium may not be evident.

Summary

A qualitative color test is described for the Grignard reagent. The test color is only shown by those organomagnesium halides having the -MgX group attached to carbon.

AMES, IOWA

[CONTRIBUTION FROM THE DRUG CONTROL LABORATORY IN COÖPERATION WITH THE COLOR LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

AZO DYES FROM ALKALOIDS OF IPECAC ROOT AND THEIR IDENTIFICATION BY MEANS OF THE SPECTROSCOPE[1]

BY S. PALKIN AND H. WALES

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In a recent paper[2] a method was outlined for the identification of phenols, based on the coupling of these substances with diazonium compounds and the measurement of the absorption spectrum maxima of the dyes thus formed. The present paper describes the application of this principle to the identification of certain ipecac alkaloids which are either phenols or amines. The proposed method identifies the cephaeline obtained from a few drops of fluid extract of ipecac in a mixture of 100 cc. of other drugs, if not unduly complicated by interfering substances.

The alkaloids of ipecac have been studied by Paul and Cownley,[3] Hesse[4] and Carr and Pyman.[5] Carr and Pyman have shown that ipecac contains five alkaloids, namely, emetine, cephaeline, psychotrine, methylpsychotrine and emetamine. According to these authors cephaeline and psychotrine are phenolic, and emetamine is probably an amine.

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[1] Read before the Division of Chemistry of Medicinal Products at the Baltimore Meeting of the American Chemical Society, April, 1925.

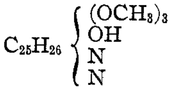
[2] Palkin and Wales, THIS JOURNAL, **46**, 1488 (1924).

[3] Paul and Cownley, *Pharm. J. Trans.*, [3] **25**, 111, 690 (1894–5); *Pharm. J.*, [4] **7**, 98 (1898).

[4] Hesse, *Ann.*, **405**, 1 (1914).

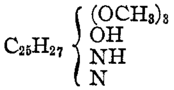
[5] Carr and Pyman, *J. Chem. Soc.*, **105**, 1591 (1914); **111**, 419 (1917).

Emetine is a methyl derivative of cephaeline, and psychotrine differs from cephaeline by the absence of an imino group. The relationship of the three principal alkaloids is as follows.



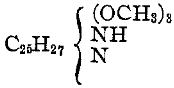
$C_{25}H_{26}\left\{\begin{matrix}(OCH_3)_3\\ OH\\ N\\ N\end{matrix}\right.$

Psychotrine



$C_{25}H_{27}\left\{\begin{matrix}(OCH_3)_3\\ OH\\ NH\\ N\end{matrix}\right.$

Cephaeline



$C_{25}H_{27}\left\{\begin{matrix}(OCH_3)_3\\ NH\\ N\end{matrix}\right.$

Emetine

The relation between the absorption spectra and the constitution of the alkaloids of ipecacuanha was studied by Dobbie and Fox.[6] This study was confined to the behavior of the alkaloids and not the dyes derived from them, in the ultraviolet region, and brought out a constitutional similarity to catechol.

The method of dye formation described elsewhere,[2] was applied to the alkaloids as follows.

Formation of the Dyes

A small portion of the acid solution of the alkaloid (2 or 3 mg.) was cooled with ice and 2 drops of a solution of *p-nitrodiazobenzene* were added. A solution of potassium hydroxide was then added, drop by drop, until the dye was formed. The solution was then acid with hydrochloric acid, the dye acid serving as its own indicator, and the free dye acid was extracted several times, first with chloroform and finally with amyl alcohol. The two extracts were evaporated to dryness on the steam-bath, and portions of each residue were dissolved in alkaline solutions of (1) acetone, (2) alcohol and (3) water[7] and examined spectrophotometrically. In all cases the chloroform portion was practically insoluble in dilute aqueous alkali. The chloroform extract of the dye is variable, both in quantity and color, as shown by the spectroscopic measurements, and seems to be produced at the expense of the amyl alcohol fraction. Where an excess of alkaloid was used in the coupling, only a trace of chloroform-soluble dye acid was obtained; conversely, with an excess of diazo reagent this fraction increased with a corresponding decrease in the amyl alcohol portion. The fraction insoluble in chloroform but soluble in amyl alcohol represents the dye characteristic of the alkaloid. In all cases, however, the chloroform extraction must be carried out to free the dye from interfering colors. The results obtained on the chloroform fractions, although without particular significance, are given in all cases for completeness.

Purification of Cephaeline and Emetine

A sample of cephaeline hydrochloride obtained from a reputable manufacturer was found to contain non-phenolic alkaloid. Likewise, a sample of emetine hydro-

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[6] Dobbie and Fox, *J. Chem. Soc. Trans.*, **105**, 1639 (1914).

[7] The solvents were made by adding 2 drops of a 20% solution of potassium hydroxide in methyl alcohol to 20 cc. of acetone, or ethyl alcohol; with water, aqueous alkali was used.

chloride which complied with the U. S. P. tests, upon treatment with diazo reagent yielded a noticeable quantity of dye. As emetine is neither a phenol nor an amine, there was no reason to believe that it would couple with diazonium salt. As it seemed probable, therefore, that the formation of this dye indicated an impurity in the emetine hydrochloride, purification was effected as follows. A sample of the hydrochloride of the alkaloid was dissolved in water, the solution was made alkaline with ammonia, and the alkaloids were shaken out completely with ether. The ether solution was then extracted with 2.5% potassium hydroxide solution to remove the phenolic alkaloids, to a point where a test showed complete extraction. This extract (aqueous) was then made slightly acid with sulfuric acid and used in subsequent dye tests. The ether was washed several times with water to free it from alkali and evaporated to dryness. This non-phenolic alkaloid residue from the ether was then dissolved in a little dil. sulfuric acid. In the case of the cephaeline hydrochloride this portion was very small but in the case of the emetine hydrochloride it constituted the greater portion of the alkaloid.

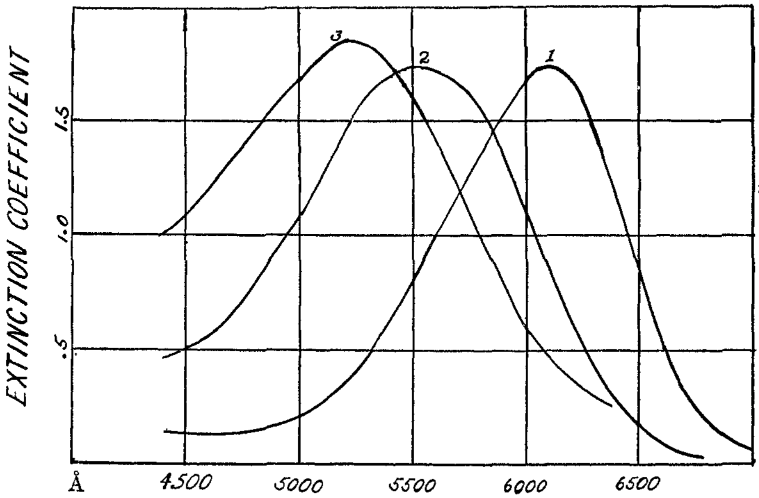

Fig. 1.—*p*-Nitrobenzene-azo-cephaeline.

Examination of the Dyes

Cephaeline.—When the phenolic portion obtained from the cephaeline hydrochloride was coupled as described above, an intense blue-purple color was produced. The dye acid was liberated, extracted, etc., as described above. The color and absorption spectrum maxima are recorded in Table I and the absorption spectra curves are shown in Fig. 1. The non-phenolic portion produced only a trace of dye, probably because the cephaeline had been incompletely removed from the ether by the alkaline extraction.

Emetine.—When treated with the coupling reagent, the phenolic portion from the emetine hydrochloride produced little dye, which was identified as being derived from cephaeline (Table I). Upon similar treatment the non-phenolic portion of the alkaloid yielded the merest trace of a dye, the absorption spectrum measurements of which indicated that the emetine hydrochloride was not entirely free from cephaeline.

Psychotrine.—An attempt was made to obtain psychotrine from fluid extract of ipecac in accordance with a method of Hesse,[4] but the alkaloid obtained gave a dye apparently identical with that prepared from cephaeline. In view of the very small quantity of psychotrine normally present in ipecac, a dye test of this compound can have no diagnostic value. The strong dye reaction of the principal phenolic alkaloid, cephaeline, would tend to obscure the effect of the dye from psychotrine.

Emetamine.—When an ether solution of the total alkaloids of ipecac is extracted with aqueous potassium hydroxide until the phenolic alkaloids have been completely removed, the non-phenolic alkaloids remaining in the ether, with *p*-nitrodiazobenzene, yield a powerful dye, blue-green in acetone, blue in alcohol, and royal purple in water, with an absorption spectrum entirely different from that shown by the dye prepared from cephaeline.

Emetamine is present in ipecac in relatively small proportion. It was obtained by Carr and Pyman from the mother liquor remaining after the separation of emetine (as the hydrobromide) from the non-phenolic ipecac alkaloids.[5] It is not commercially available and no sample of it could be bought for this investigation. The mixed non-phenolic ipecac alkaloids were converted into the hydrobromides, and most of the emetine hydrobromide was removed by crystallization. The emetine thus obtained yielded only a trace of dye, and the mother liquor retained practically all the dye-forming ingredient originally present in the mixture. The azo dye derived from this ingredient has a tinctorial power as great as or greater than that of the azo dye derived from cephaeline. There appears to be little doubt that this non-phenolic alkaloid is that designated by Carr and Pyman as emetamine. As emetamine is not an article of commerce, its presence with cephaeline in a medicinal product may be regarded as evidence of the presence of ipecac extractive.

Identification of Ipecac in Its Preparations

The fluid extract may be used to illustrate the method by which the procedure described would be applied to the identification of ipecac in its preparations. A convenient quantity of fluid extract was diluted to three or four times its original volume, sulfuric acid added to bring the solution to 0.5 N strength and any non-alkaloidal phenol that might be present extracted with ether. The acid solution was treated with an excess of ammonia, and the alkaloids were completely extracted with ether. The phenolic and non-phenolic alkaloids in the ether were then separated by complete extraction of the phenolic portion with potassium hydroxide solution. The solution of phenolic alkaloid was then acidified.

The ether solutions of the non-phenolic and phenolic alkaloids were evaporated, and the alkaloidal residues were converted into dyes. When examined spectrophotometrically the dyes soluble in amyl alcohol yielded the absorption-spectra data given in Table I and shown in Figs. 1 and 2.

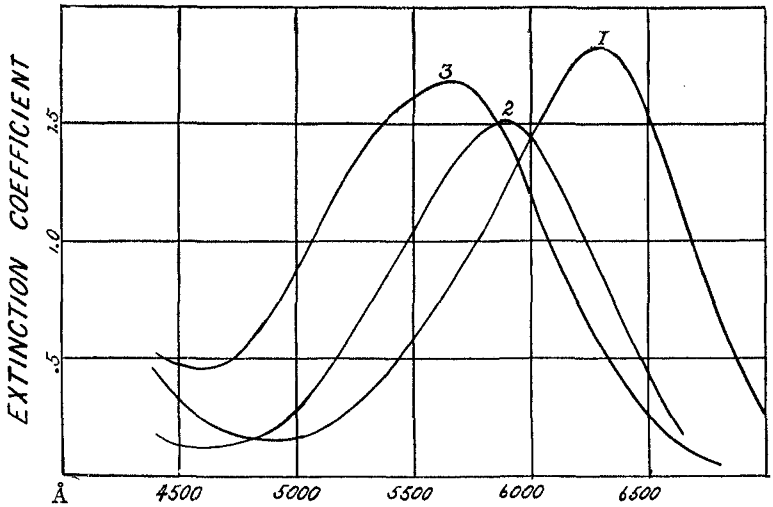

Fig. 2.—*p*-Nitrobenzene-azo-emetamine.

TABLE I

IPECAC ALKALOIDS

Solvent for dye examination	Phenolic fraction of alkaloid		Non-phenolic fraction of alkaloid	
	Chloroform extract of dye[a] Å.	Amyl alcohol extract of dye Å.	Chloroform extract of dye[a] Å.	Amyl alcohol extract of dye Å.
		EMETINE		
Acetone	5500	6100	5450	Trace
Alcohol	5150	5550	5100	Trace
Water	Insol.	5250	Insol.	Trace
		CEPHAELINE		
Acetone	5700	*6100*	5450	6100
Alcohol	5300	*5550*–	5150	5550
Water	..	*5250*	Insol.	..
		CRUDE DRUG[b]		
Acetone	Trace	*6100 (blue)*	5700 6050	*6300 (blue-green)*
Alcohol	Trace	*5525 (purple)*	5500	*5900 (dark blue)*
Water	Trace	*5250 (red)*	Insol.	*5700 (royal purple)*
		U. S. P. FLUID EXTRACT OF IPECAC		
Acetone	5500	*6100 (blue)*	5500	*6300 (blue-green)*
Alcohol	5300	*5525 (purple)*	5300	*5900 (dark blue)*
Water	Insol.	*5250 (red)*	Insol.	*5700 (royal purple)*

[a] Readings of chloroform-soluble dye varied by 100 Å.

[b] Rio and Carthagena varieties gave identical results for corresponding portions.

The Alkaloids of Rio and Carthagena Ipecac

The Pharmacopœia recognizes two varieties of ipecac, the dried root of *Cephaelis Ipecacuanha* (Brotero) A. Richard, known in commerce as Rio Ipecac and *Cephaelis acuminata* Karsten, known in commerce as Carthagena ipecac. In order to determine whether or not the method of examination proposed would show any differences in the composition of the two varieties, a sample of each was extracted and the total alkaloids were separated and examined. As shown in the table, the dye derivatives obtained from the corresponding alkaloids of the two varieties of the drugs are identical.

The authors wish to express their appreciation to Mr. A. G. Murray for helpful suggestions and criticisms in the preparation of this paper.

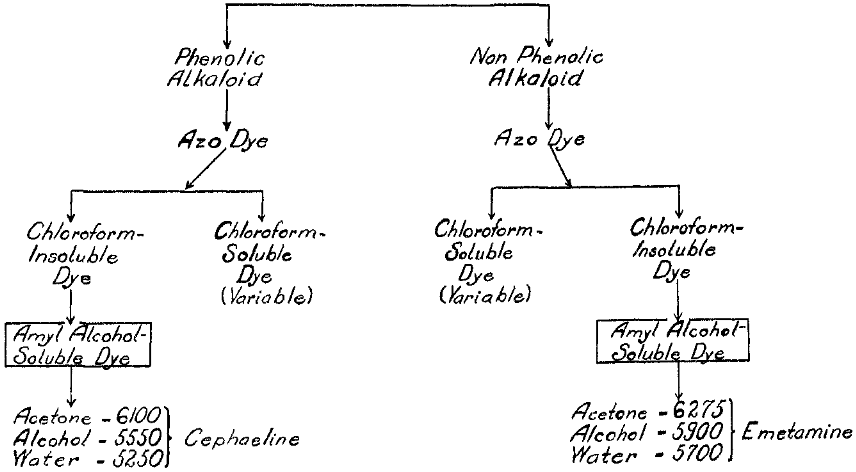

Fig. 3.—Total ipecac alkaloids.

Summary

A method for the identification of phenols described in a recent paper has been extended to apply to certain alkaloids of ipecac. Azo dyes from these alkaloids of ipecac have been prepared and measurements made of their absorption spectra in three different solvents. A number of characteristic curves have been thus obtained for cephaeline and emetamine, the absorption spectrum maxima of which can be used for their positive identification even in the presence of a considerable quantity of other drugs, if not unduly complicated by interfering substances. A diagrammatic summary is shown in Fig. 3.

WASHINGTON, D. C.