Anal. Calcd. for $C_{23}H_{14}N_4O_7S$: N, 11.5. Found: N, 11.8.

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Paper Chromatography of Anthraquinone Pigments

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The paper partition chromatography developed by Consden, Gordon and Martin[1,2,3] has been very usefully applied in the course of studies on the micro-determination of amino acids and carbohydrates. The separation of organic acids,[4] anthocyanins,[5] purines[6] and flavonoid pigments[7] has also been carried out by this method.

In the course of studies on the micro-determination of the principles of crude drugs, we have applied this method to the separation and identification of anthraquinone pigments.

TABLE I

Rf-VALUES OF HYDROXYANTHRAQUINONES

(97% methyl alcohol saturated benzine as solvent, at 24–25°)

Subst.	Positions of substituents in anthraquinone nucleus	Rf[a]	Color of spot
Chrysophanol	4,5-Dihydroxy-2-methyl	0.92	Orange
Physcion	4,5-Dihydroxy-7-methoxy-2-methyl	.89	Orange
Quinizarin	1,4-Dihydroxy	.89	Purple
2-Methylquinizarin	1,4-Dihydroxy-2-methyl	.92	Purple
Emodin	4,5,7-Trihydroxy-2-methyl	.52	Pink
Rubiadin	1,3-Dihydroxy-2-methyl	.49	Orange-yellow
Aloe-emodin	4,5-Dihydroxy-2-hydroxymethyl	.15	Orange
Rhein	4,5-Dihydroxy-2-carboxyl	0	Orange
Alizarin	1,2-Dihydroxy	.04	Violet
Endocrocin	4,5,7-Trihydroxy-2-methyl-3-carboxyl	0	Pink
Purpurin	1,3,4-Trihydroxy	0.03	Purple
Dihydroxymethylanthraquinone	1,5-Dihydroxy-2-methyl	.92	Orange
	1,8-Dihydroxy-2-methyl	.92	Orange
Tetrahydroxyanthraquinone	1,3,5,7-Tetrahydroxy-2,6-dimethyl	.02	Orange
	1,3,6,8-Tetrahydroxy	.01	Orange-pink
Rhodocladonic acid	1,3,6,8-Tetrahydroxy-2-hydroxymethyl-7-carboxylic acid methyl ester	0	Orange
Anthragallol	1,2,3-Trihydroxy	0	Gray
Rufigallic acid	1,2,3,5,6,7-Hexahydroxy	0	Grayish-violet

[a] Rf values were measured to the leading edge of the spot.

In this preliminary report the procedures for separation, identification and determination of Rf-values of several synthetic and natural hydroxyanthraquinones are described. The relationship between the Rf-values and their structures is also discussed. We have found that benzine (b. p. 45–70°) saturated with 97% methyl alcohol is a very satisfactory solvent[8] for one-dimensional ascending separation of these compounds which are very sparingly soluble in water. The acetone solution of the mixture of authentic specimens of hydroxyanthraquinones spotted on a filter paper strip (2 cm. × 40 cm., Toyo-Filter Paper No. 2) was dried prior to the development. The development was carried out in a well closed glass cylinder (50 cm. in height and 12 cm. in diameter), for five to six hours at 24–25°, when the solvent ran about 30 cm. After air-drying, the developed paper strip was sprayed with 0.5% methyl alcoholic magnesium acetate solution and heated at 90° for five minutes. Distinct orange-red, purple or violet colored spots, depending on the position of hydroxyl groups in the anthraquinone nucleus, were given by this reagent.[9] Anthraquinones having at least one of the hydroxyl groups in the α-position develop with the reagent. Compounds which contain two in the 1,3-position, *i. e.*, emodin, chrysophanol or aloe-emodin, give an orange-red or pink color; those with two in the 1,4-position, *i.e.*, quinizarin, produce a purple; and those with two in the 1,2-position, *i.e.*, alizarin, exhibit a violet color.

This color reaction is specific, stable and very sensitive so that the very faint yellowish color on the paper strip given by a trace of hydroxyanthraquinone becomes clearly visible when the faint yellowish color is converted to a red or purple color by spraying with the magnesium

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(1) R. Consden, A. J. P. Martin and A. H. Gordon, *Biochemical J.*, **38**, 224 (1944).

(2) A. J. P. Martin, *Ann. New York Acad. Sci.*, **49**, 249 (1948).

(3) R. Consden, *Nature*, **162**, 359 (1948).

(4) B. T. Overell, *ibid.*, **160**, 87 (1947).

(5) E. C. Bate-Smith, *ibid.*, **161**, 835 (1948).

(6) E. Vischer and E. Chargaff, *J. Biol. Chem.*, **168**, 781 (1947).

(7) S. H. Wender and T. B. Gage, *Science*, **109**, 287 (1949).

(8) The temperature at which benzine is saturated with 97% methyl alcohol, which in this experiment was 19°, appears to have some effect on the Rf-values.

(9) S. Shibata, *J. Pharm. Soc. Japan*, **61**, 103 (in German) (1941).

acetate reagent. From the shade of the spot a suggestion as to the position of hydroxyl groups may be obtained.

Chrysophanol gives a very high Rf-value, whereas emodin, having one more hydroxyl group in the 7-position, gives a lower value and compounds such as endocrocin (emodin-3-carboxylic acid), rhein and aloe-emodin, which have carboxyl or carbinol groups in their molecules, show very little movement. Quinizarin which possesses two hydroxyl groups in the 1,4-positions moves relatively more easily than emodin; alizarin, containing 1,2-hydroxyl groups in its molecule, gives a very low rate of movement. Methylation of the hydroxyl in the 7-position of emodin shifted its spot toward that of crysophanol.

Estimation of the purgative anthraquinone crude drugs by this method is now in progress.

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The Separation of Americium and Curium from the Rare Earth Elements

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Introduction

In connection with the general problem of determining the nuclear and chemical properties of the elements americium and curium, it is very desirable to have fast and efficient methods of isolating them from the other elements. The separation of americium and curium from the rare earth elements presents one of the most difficult problems encountered in this work, and consequently considerable time has been devoted to achieving a satisfactory separation procedure.

As is the case for the rare earth elements, the only thermodynamically stable oxidation state of americium and curium in aqueous solution is the tripositive; and since their crystal radii overlap those of the light rare earth elements, being about the same as neodymium, the solubilities of their compounds are very similar to those of the rare earths. The only reasonably satisfactory group separation of americium and curium from the rare earths known when the present work was undertaken was the procedure known as "the fluosilicate precipitation."[1,2] This procedure, which involves partial precipitation of lanthanum fluoride carrier, has the serious drawback of only giving fractionation of the lanthanides and actinides and hence requires numerous repetitions of a relatively low yield step to achieve even a moderately satisfactory separation.

For many of the nuclear experiments contemplated a good yield and a very complete separation of americium and curium from rare earth fission products and from each other was necessary, but because of the relatively long half-lives of the isotopes involved, time was not a major consideration. For work with isotopes of short half-life a method which would give a very rapid and complete separation from the rare earths and some fractionation of americium and curium had to be found. Because a promising method of attack was at hand, a solution to the first problem was sought first, and very fortunately in the course of its solution the direction of a satisfactory solution to the second problem was indicated.

The very successful separation of the rare earth elements from each other by selective elution from columns of cation exchange resins with ammonium citrate solutions[3] led Cunningham and co-workers[4] to try this method for the separation of americium and curium with equally good results. Because of this success the elution with citrate was taken as a starting point for further investigation.

Experimental

In order to establish the elution behavior of americium and curium and the rare earth elements under our experimental conditions a run was made at room temperature using 0.25 *M* citric acid solution adjusted to *p*H 3.05 with concentrated ammonium hydroxide. The elution curve is shown in Fig. 1.

In this experiment and all those described below tracer amounts were used and the isotopes employed, namely, Am^{241}, Cm^{242}, Ce^{144}, Pm^{147} and Lu^{172}, were identified by their radiation characteristics. In this and the following figures the ordinate is given as counts per minute per drop, where the counting was done with a Geiger counter for all except americium and curium whose alpha particles were counted. The data are given in this direct manner since in tracer experiments of this type the relative amounts are unimportant and no purpose is served by calculating the number of atoms on an absolute basis through the use of the half-lives and counting efficiencies of the isotopes concerned. The abscissas are given in arbitrary units since the actual volume of solution necessary to elute a given element will vary with the particular "batch" of resin used as well as with the column size. With our resin and columns 1 unit is about 0.030 cc. It will be noted that americium is eluted at the same rate as promethium so that separation from this fission product is not obtained by this method.

There was no *a priori* reason to believe that equally good separation of americium and curium could not be effected with eluting solutions other than ammonium citrate. In addition the possibility existed that due to more subtle differences between the actinides and the rare earth elements, the relative rate of elution of americium and curium with respect to the rare earths might be shifted so that with a different eluting solution a rare earth other than promethium would be eluted at the same rate as americium. Thus, although the time required might be long, a complete decontamination of americium and curium from the rare earths might be effected by eluting with one solution and then placing the material on another column and eluting with a different solution.

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(1) S. G. Thompson, R. A. James, L. O. Morgan and I. Perlman, National Nuclear Energy Series, Plutonium Project Record, Vol. 14B, "The Transuranium Elements: Research Papers," Paper No. 19.1 (McGraw-Hill Book Co., Inc., New York, N. Y., 1949).

(2) R. A. James, Ph.D. Thesis, University of California, 1948.

(3) The history of the development of ion exchange separations of the rare earth elements within the Manhattan Project is given by W. C. Johnson, L. L. Quill and F. Daniels, *Chem. Eng. News*, **25**, 2494 (1947).

(4) B. B. Cunningham, E. R. Tompkins and L. B. Asprey, unpublished work.