strict analogs, octadecyl alcohol and octadecyl mercaptan, the same is true. Molecular weight seems to be of less importance than structure for with the two isomers stearic acid and ethyl palmitate, considerable differences in adsorption were apparent. Moreover, octadecene, the lowest molecular weight substance of the group was able to displace several substances of higher molecular weight.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF OKLAHOMA]

The Isolation and Purification of Morin on an Ion-Exchange Resin[1,2]

BY QUENTIN L. MORRIS, THOMAS B. GAGE AND SIMON H. WENDER

Amberlite IRC-50(H) synthetic cation exchange resin has been utilized for the isolation and purification of morin (2′,3,4′,-5,7-pentahydroxyflavone) from aqueous extracts of the heartwood of *Chlorophora tinctoria*. The morin is further purified, after elution from the column with ethyl alcohol, by recrystallization from acetic acid, conversion to the potassium salt and re-adsorption on a fresh column of Amberlite IRC-50(H). The morin, thus prepared, compares favorably in its properties with authentic morin obtained by the more involved classical procedures. The method offers a practical low-cost procedure for the preparation of morin or other flavonoid pigments from plant extracts.

Introduction

Rekers and Fields[3] recently reported the successful use of morin, 2′,3,4′,5,7-pentahydroxyflavone, in the prevention of mortality in dogs from mid-lethal doses of total-body X-radiation. Analyses elsewhere[4] and in this Laboratory have revealed that the morin content of the Eastman Technical grade used by Rekers and Field was not in excess of two per cent. This has led to efforts to obtain pure morin in order to evaluate properly its protective effect in radiation sickness.

Bonner[4] and Haley and Bassin[5] have recently reported methods for the isolation and purification of morin. These methods involve either vacuum sublimation or the evaporation of considerable quantities of water in the isolation procedure.

For the preparation of morin in quantities of 50–100 g., sublimation or evaporation at reduced temperature and pressure requires a considerable investment in time and equipment. Furthermore, in the purification of morin obtained by aqueous extraction of *Chlorophora tinctoria*[6] wood chips followed by evaporation of the extract, we have found that a significant quantity of the product was in the form of the potassium and calcium salts.

The procedure to be described in this paper does not require the use of vacuum sublimation, and, by passage through a cation exchange resin bed, sharply reduces the amount of contamination due to metallic ions. This is the first reported instance of the use of ion exchange resins for the isolation, purification and recovery of morin.

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(2) Presented before the Sixth Southwest Regional Meeting of the American Chemical Society, December 7–9, 1950, San Antonio, Texas.

(3) P. E. Rekers and J. B. Field, *J. Clin. Invest.*, **28**, 746 (1949).

(4) John F. Bonner, Jr., Report UR-111, University of Rochester Atomic Energy Project, Rochester 20, New York.

(5) Thomas J. Haley and Murray Bassin, Report UCLA-73, University of California at Los Angeles Atomic Energy Project, P. O. Box 31, Beverly Hills, California.

(6) H. Hlasiwetz and L. Pfaundler, *Ann.*, **127**, 352 (1863).

Experimental

One kilogram of ground heartwood of the *Chlorophora tinctoria* tree was extracted four times with 12-gal. portions of distilled water. Each extract was boiled for 2 hr. and then filtered through flannel bags. The cooled filtrate was then passed through 2 in. × 48 in. Pyrex columns packed with Amberlite IRC-50(H) cation exchange resin. Four such columns were used. The resin bed had been previously washed with 0.2 *N* hydrochloric acid, backwashed with distilled water and downwashed with additional distilled water until the washings were neutral.

Approximately 12 gal. of the red-brown extract was passed through each column. The filtrate from the columns was only slightly less colored. The resin bed gradually assumed a yellow-brown tint as the solution passed through. The columns were next washed with distilled water until the filtrate was clear (3–4 l.). The adsorbed material, including morin and maclurin, was then eluted with 95% ethyl alcohol. Each column required about 1 l. of alcohol. The alcohol wash was followed by distilled water in order to flush the last of the alcoholic solution through the resin bed.

Some yellow-brown material moved down the column just in advance of the alcohol–water interface. This material precipitated on leaving the column. It was discarded since it gave negative tests for flavonoid material. The alcoholic filtrates from the four columns were combined and concentrated to approximately 250–300 ml. Considerable yellow-brown solid material precipitated during the concentration. An equal volume of water was added to the concentrate and the solution set in the refrigerator overnight to allow further precipitation to take place; yield 26.4 g. or 2.64% of crude morin. Most of the maclurin remained in solution.

The crude morin was recrystallized from 900 ml. of 60% acetic acid solution; yield 12 g. or 1.2%. The last traces of maclurin were removed at this point. The dried morin was dissolved in a minimum quantity of 95% ethyl alcohol and 15 g. of solid potassium acetate added. A bright yellow precipitate of the potassium salt of morin separated at once. The potassium salt was suspended in approximately 20 l. of distilled water and a few drops of potassium hydroxide solution were added to complete solution. The *p*H of the resulting solution was approximately neutral.

The solution of the potassium salt was then passed through two fresh columns of Amberlite IRC-50(H) resin in order to decompose the complex. The potassium was exchanged for hydrogen ion and the adsorbed morin was then eluted with ethyl alcohol. Concentration of the alcoholic solution at reduced pressure and subsequent addition of water yielded pale yellow morin; yield 9.5 g. or 0.95%.

Paper partition chromatography[7] of an alcoholic solution of the final product revealed no contamination by other flavonoid or other visible or fluorescent pigments. Mixed chromatograms of this morin with an authentic sample of pure morin prepared by vacuum sublimation techniques[4] produced only one pigment zone. The melting point of the final product (289–290° dec.) was not depressed when this material was mixed with authentic morin.

Discussion

Considerable swelling of the resin bed occurs when the alcohol replaces the water on the column. The resin bed expands vertically in columns of 2 in. diameter and thus no danger of breakage is involved.

Morin has a strong affinity for many metal ions. The ash content of samples of morin prepared by the method described in this paper ranged from 0.1 to 0.2 per cent. Further passes through a cation exchange resin will reduce the ash content even more. Morin prepared from the same starting material by non-ion exchange methods had an ash content of 1 per cent. The latter method involved concentration of an aqueous extract, recrystallization from aqueous alcohol solution, precipitation of the potassium salt and recrystallization from 60% acetic acid.

The use of an ion exchange resin for the isolation and purification of morin and other flavonoid compounds appears to be a practical method for both large and small scale laboratory preparations.

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

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Absorption Spectra of Some N-Substituted *p*-Aminotriphenylmethyl Ions

BY HAROLD WALBA AND GERALD E. K. BRANCH

This paper shows the spectra of the quinoidal ions of some *p*-aminotriphenylcarbinols. These ions have been chosen so that in each case one can find in the literature the spectrum of an ion differing only in the presence of a *p*-$NH(CH_3)_2^+$ group. The *p*-aminotriphenylcarbinols have also been chosen so that in one case the terminal groups have no resonance interaction with amino nitrogen, but in the other various degrees of resonance interaction between the nitrogen atom and its terminal groups exist. This series has been obtained by using *p*-dimethylaminotriphenylcarbinol as the example in which there is no resonance interaction of the terminal groups, and deriving other carbinols by replacing the dimethylaniline with methyldiphenylamine, diphenylamine, N-acetyldiphenylamine, triphenylamine, carbazole or N-acetylcarbazole. The purposes behind the choice of carbinols were (1) to measure the effect of the *p*-$NH(CH_3)_2^+$ group on the spectrum, (2) how the resonance interactions of unsaturated terminal groups affect the spectrum and (3) how they affect the effect of the *p*-$NH(CH_3)_2^+$ group.

The spectra of the quinoidal ions all have three strong bands, which we have called the first, second and third band, starting from the band with the longest wave length. The third band is very little affected by terminal unsaturated groups or by a *p*-$NH(CH_3)_2^+$ group. On the other two bands a *p*-$NH(CH_3)_2^+$ is normally hypsochromic. The resonance interaction is bathochromic on the second band. The wave length of the first band increases with that of the second band to a maximum and thereafter decreases. The separation of the first and second bands increases with the wave length of the second band to a maximum and thereafter decreases. The hypsochromic effect of the $NH(CH_3)_2^+$ group on the first band decreases as the wave length of the second band increases and eventually becomes bathochromic. The above peculiar relationships are explained on the basis of the following assumptions. (1) The first band is the *x*-band. (2) The second band is the *y*-band. (3) The labile charge is distributed over the molecule by resonance, but is chiefly on the nitrogen atom in the ion of *p*-dimethylaminotriphenylcarbinol. (4) The resonance interaction of unsaturated terminal groups tends to push the labile charge to the other end of the molecule. (5) The $NH(CH_3)_2^+$ group has the opposite effect. (6) Location of labile charge in the $C(C_6H_5)_2$ group is bathochromic on the second band. (7) Unequal distribution of the labile charge between the two ends of the molecule is hypsochromic on the first band. These assumptions have been embodied in empirical equations giving the wave numbers of the first bands and those of the first bands of the *p*-$NH(CH_3)_2^+$ derivatives as functions of the wave numbers of the second bands of the ions of the monoaminotriphenylcarbinols. Values calculated from these equations agree fairly well with those observed with one exception. The exception is $CH_3CO(C_6H_5)^+N{=}C_6H_4{=}C(C_6H_5)C_6H_4NH(CH_3)_2^+$. The suggested tautomerization is the transference of the proton from the $NH(CH_3)_2^+$ to the oxygen atom. This is similar to the *p*-nitroso derivative of dimethylaniline, where the spectrum shows that the proton shifts from the $NH(CH_3)_2^+$ group to the oxygen atom.

Tolbert and Branch[1] have measured the absorption spectra of the second ions of a set of *p,p'*-diaminotriphenylcarbinols, in which one of the amine components was dimethylaniline in each case, and the other was varied, being dimethylaniline, methyldiphenylamine, diphenylamine, triphenylamine or carbazole. In this article we are reporting the absorption spectra of the ions of the corresponding set of *p*-monoaminotriphenylcarbinols, the amine components being dimethylaniline, methyldiphenylamine, diphenylamine, triphenylamine or carbazole. The formulas of the ions are I, II, III, IV and V. Those of the second ions of the corresponding set of diamino compounds are I', II', III', IV' and V'.

(1) B. M. Tolbert and G. E. K. Branch, THIS JOURNAL, **69**, 1083 (1947).