mg. (0.4–1.0 ml. of a 5% solution) of sodium nitroprusside is used.

Effect of Sodium Hydroxide.—Figure 5 illustrates the effect of the molarity of the sodium hydroxide solution on color intensity. Maximum intensity is obtained when the sodium hydroxide concentration is 0.5–1.0 molar. However, maximum stability of color is obtained when 0.5 ml. of a one-molar solution is used.

Effect of Sodium Bicarbonate.—An amount of sodium bicarbonate greater than that of the sodium hydroxide present is necessary for full development of color. When solid sodium bicarbonate is added in place of sodium bicarbonate solution, the violet color changes to rose and fades rapidly. For maximum color stability, 1.0 ml. of one-molar solution was found to give the best results in the case of Otrivin hydrochloride. However, optimum conditions should be studied for each imidazoline under consideration. A volume up to 2.0 ml. of a one-molar sodium bicarbonate solution does not affect color intensity, but greater volumes result in less intense and less stable solutions.

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Reaction of Rauwolfia Alkaloids, Indoles, and Related Compounds to Nitrite*

By R. P. HAYCOCK and W. J. MADER

The greenish-yellow color complex resulting from the addition of sodium nitrite to an alcoholic solution of reserpine in the presence of dilute sulfuric acid has been re-examined to determine the selectivity of the reaction and the chromophore groups fundamentally responsible for the color. Its application to other rauwolfia alkaloids, indoles, and related compounds was investigated. A new indican reaction is reported for simpler indoles.

THE RAUWOLFIA ALKALOIDS have received much attention since the isolation of reserpine was reported by Mueller, Schlittler, and Bein (1). The difficulties involved in establishing a method of analysis specific for reserpine is apparent when one considers the structural complexity of reserpine and its similarity to other alkaloids found in various species of Rauwolfia. Without enumerating all procedures, mention should be made of some of the methods described for the separation and determination of reserpine in therapeutic preparations. Chromatographic techniques, both paper and column, have been used extensively (2–4). A method is described for the estimation of reserpine based on the ultraviolet absorption of 3,4,5-trimethoxybenzoic acid liberated by hydrolysis (5). A fluorometric method for the determination of reserpine in tablets and extracts of *Rauwolfia serpentina* is based on a reaction with hydrogen peroxide (6). Reserpine has been determined by ultraviolet absorption and colorimetrically and fluorometrically after heating with strong acids (7). Other colorimetric procedures include: the formation of a chloroform soluble complex with bromophenol blue (8), a color complex using a conventional reagent, vanillin (9), and a colorimetric technique using sodium nitrite in the presence of sulfuric acid (10).

Notwithstanding the abundance of analytical procedures, the application of existing methods presented certain difficulties and inconveniences. Consequently, the colorimetric Szalkowski-Mader nitrite technique was re-examined. The authors indicate that the greenish-yellow colored complex resulting from the addition of sodium nitrite to a methanolic solution of reserpine in the presence of sulfuric acid is specific and does not react with alstonine, rauwolscine, sarpagine, yohimbine, and deserpidine. Although no fundamental changes were made, Banes (11, 12) and coworkers proposed improvements to the application of the method to reserpine and to reserpine-rescinnamine group alkaloids in pharmaceutical

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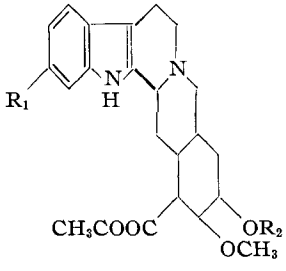
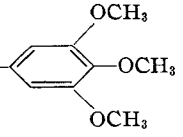
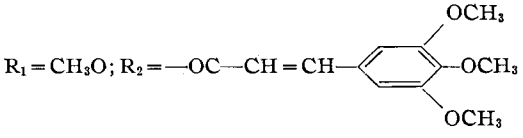
preparations. This modification, according to Banes, corrects for 3-dehydroreserpine a degradation product of reserpine and other substances absorbing light at 390 mμ prior to nitrite treatment. However, the selectivity of the reaction and the chromophore groups fundamentally responsible for the color has not been adequately investigated. This led to the study of a number of alkaloids, indoles, and related compounds, to determine the effect of variations in the molecule upon the colorimetric nitrite procedure, on the assumption that this might find application in the elucidation, or corroboration, of the structure of alkaloids from various species of Rauwolfia.



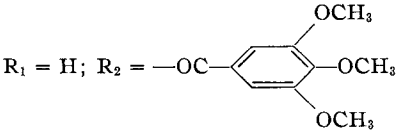
$R_1 = CH_3O$; R_2 =



Reserpine



Rescinnamine



Deserpidine

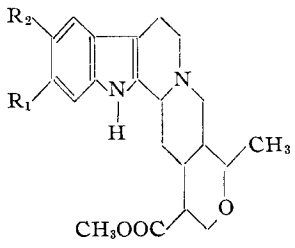
Figure 1.

EXPERIMENTAL

Solutions.—(*a*) Indole solutions: Dissolve 25.0 mg. of the indole in 100 ml. of methanol. Dilute 4.0 ml. to exactly 100 ml. with methanol. The diluted indole solutions contain 10 mcg. of the indole per ml. (*b*) Carboline solutions: Dissolve 5.0 mg. of the carboline in 100 ml. of methanol. Dilute 10.0 ml. to exactly 100 ml. with methanol. The diluted carboline solutions contain 5 mcg. of the carboline per ml. (*c*) Alkaloid solutions: Dissolve 5.0 mg. of the alkaloid in 100 ml. of methanol. Dilute 20 ml. to exactly 50 ml. with methanol. The diluted alkaloid solutions contain 20 mcg. of the alkaloid per ml. (*d*) Sulfuric acid solutions 1 *N:* Dissolve about 30 ml. of sulfuric acid in one liter of water. (*e*) Aqueous sodium nitrite solution 0.3%. (*f*) Aqueous sulfamic acid solution, 5%. Prepare fresh every two or three days.

PROCEDURE

The procedure is essentially that of Szalkowski and Mader (10) as modified by Banes and associates (11). Transfer duplicate 10.0-ml. aliquots equivalent to 100 mcg. of the indoles, 50 mcg. of the carbolines, and 200 mcg. of the alkaloids to 150 x 18 mm. test tubes. To each tube add 3.0 ml. of sulfuric



$R_1 = CH_3O$; $R_2 = H$
Reserpinine

$R_1 = CH_3O$; $R_2 = CH_3O$
Reserpiline

$R_1 = H$; $R_2 = CH_3O$
Aricine

Figure 2.

acid solution, to one of the duplicate tubes add 2.0 ml. of sodium nitrite solution, and to the other duplicate tube add 2.0 ml. of distilled water. Mix the contents of each tube and allow to stand for one hour. Add to each tube 0.5 ml. of sulfamic acid solution, mix, and filter through paper. After stabilization of the solutions, determine the spectral characteristics with a suitable recording spectrophotometer in matched 1-cm. cuvettes relative to the sample blank containing no nitrite.

DISCUSSION

As indicated previously, the authors of the nitrite procedure for reserpine claimed that sarpagine, rauwolscine, alstonine, yohimbine, and deserpidine, did not react. An examination of the structure of these alkaloids indicated that the common structural difference from reserpine was the absence of an 11-methoxy group. Consequently, the spectral characteristics of other tertiary indole alkaloids were investigated to verify these observations. Rescinnamine (Fig. 1), which differs from reserpine only in that the esterfied alcoholic function is trimethoxycinnamic acid, instead of trimethoxybenzoic acid, produced chromogenic values approximately equal to those of reserpine. Reserpinine (Fig. 2), a tertiary indole with a heterocyclic ring *E*, and possessing an 11-methoxy group, also formed the greenish-yellow complex. In order to determine the effect of two methoxy groups in ring *A*, reserpiline, which possesses a 10,11-dimethoxy group, was examined. The color complex showed essentially

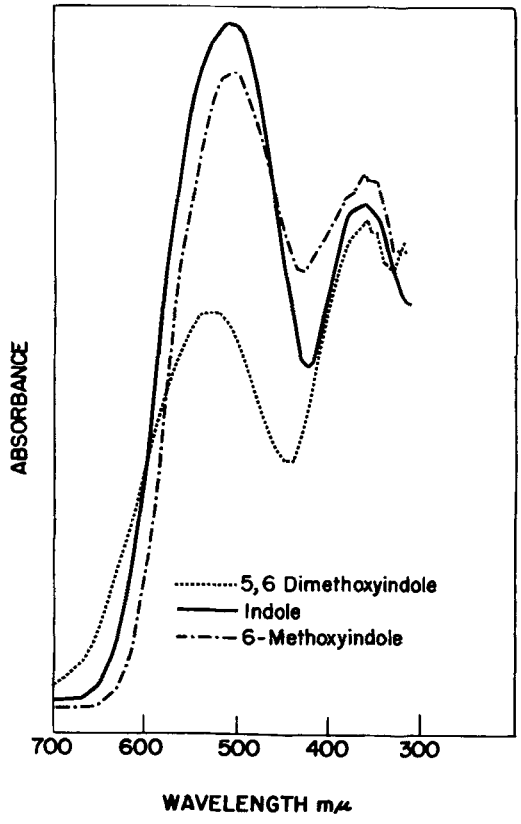


Fig. 3.—Absorption curves of nitrite treated indoles.

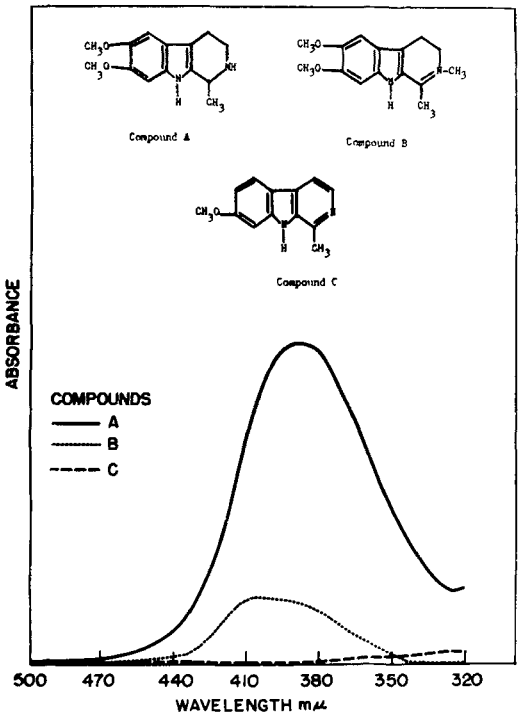
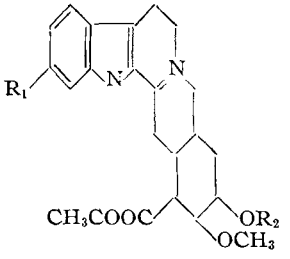
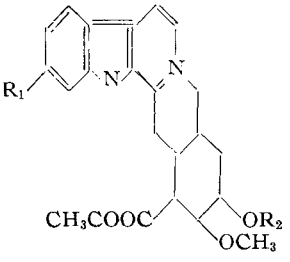


Fig. 4.—Absorption curves of nitrite treated carbolines.

the absorption of reserpinine. Aricine, which differs from reserpinine only in the position of the methoxy group, which in this alkaloid is in the 10 position, does not react. It is of interest to note that aricine develops a weak reaction on



3 Dehydroreserpine



Tetra Dehydroreserpine

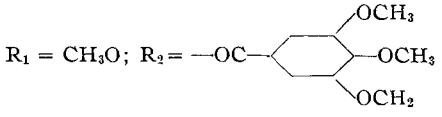


Figure 5.

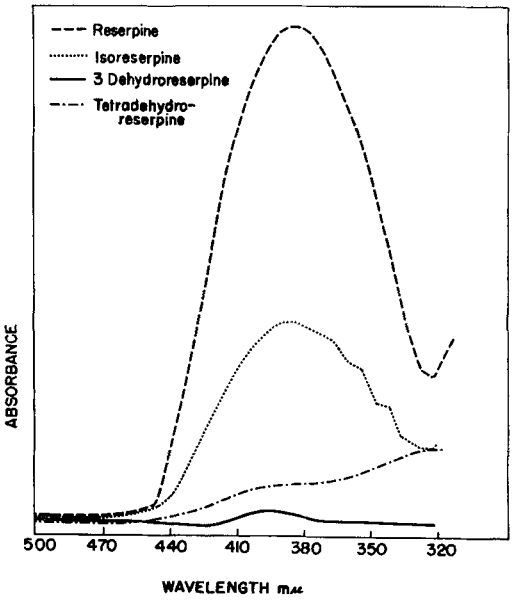


Fig 6.—Absorption curves of nitrite treated reserpine and degration products.

standing several hours. Raunescine, which also lacks an 11-methoxy group, did not yield the characteristic nitrite color. It appeared probable from the before mentioned data that an 11-methoxy group was one of the underlying groups necessary in the development of the greenish-yellow nitrite color.

In order to determine other groups necessary for the reaction, a number of indoles and related compounds were studied. When the behavior of indoles was examined as shown in Fig. 3, it was found that the addition of dilute sulfuric acid-sodium nitrite reagent to an alcoholic solution of indole, 6-methoxyindole, and 5,6-dimethoxyindole produced a pink color, rather than the greenish-yellow colow characteristic of reserpine. The effect of the concentrations of sulfuric acid and sodium nitrite, and the effect of time and temperature on the intensity and stability of the pink colored complex was not examined. However, the color appears to be stable and presumably it is a new indican reaction. The addition of groups at the 2-position reduces the color intensity displayed by the other indoles unsubstituted in that position.

Finally, the nitrite spectra of a series of β-carbolines was examined. These results are shown in Fig. 4. Compound *A*, 1,2,3,4-tetrahydro-6,7-dimethoxy-1-methylβ-carboline, which is similar to the *ABC* ring skeleton of reserpine, shows the characteristic spectrum of nitrite treated reserpine with a maximum at 390 mμ. However, compounds *B* and *C* or 6,7-dimethyl-1-methyl-3,4-dihydrocarboline and harmine which contain unsaturation in ring *C*, did not form the greenish-yellow color phenomenon. This led to the conclusion that the *ABC* ring structure of reserpine is a necessary adjunct to the formation of the color complex.

Although Banes (12) and co-workers observed that reserpine could be determined in the presence of its oxidative degradation products, no data was reported on the application of the nitrite procedure to pure 3-dehydroreserpine and tetradehydroreserpine (Fig. 5). This suggested a further study of these oxidative degradation products to ascertain whether or not the color phenomenon develops upon treatment with nitrite reagent. An examination of the absorption obtained by treating alcoholic solutions of 3-dehydroreserpine and tetradehydroreserpine with nitrite is negative and is shown in Fig. 6. This is essentially in agreement with the negative results obtained with the unsaturated β-carbolines noted previously, and substantiates the observation of Banes that reserpine can be determined in the presence of oxidation products by the nitrite procedure. Isoreserpine, which is also relatively inactive, exhibits chromogenic absorbance but it is considerably less than that of reserpine as can be seen in Fig. 6.

In earlier attempts to reproduce the spectral curves for nitrite treated reserpine, and other alkaloids, we noted a peculiar absorption pattern in the range 330–380 mμ unlike that reported by Szalkowski and Mader. Subsequent investigation revealed that this irregular absorption resulted from excess sodium nitrite as indicated in Fig. 7. Improved results were obtained by filtering the reaction mixture through filter paper, but in the case of nonreactive alkaloids and indoles, the addition of sulfamic acid directly to the reaction medium is required to remove excess nitrite and eliminate misleading results.

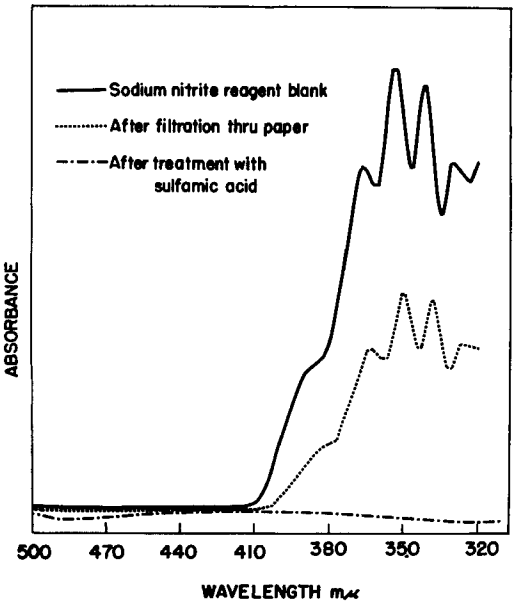


Fig. 7.—Absorption curves of sodium nitrite.

CONCLUSIONS

1. It has been established that the 7-methoxy β-carboline group, i. e., the 11-methoxy group and the *AB* and *C* ring skeleton of reserpine, is the functional group responsible for the greenish-yellow complex obtained by reacting an alcoholic solution of reserpine with sodium nitrite in the presence of dilute sulfuric acid.

2. On the basis of the formation of a greenish-yellow color with nitrite, the presence of an 11-methoxy group in alkaloids may be ascertained.

3. On the basis of sensitivity, selectivity, and reproducibility, the nitrite technique is the preferred procedure for determining reserpine.

4. A new indican reaction is reported for some indoles.

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