antagonism was moderate and was not dose related. The depressor response to McN-A-343 was enhanced by the low doses of cocaine.

DISCUSSION

The results of this study indicate postsynaptic anticholinergic activities of imipramine and methylphenidate. In spite of the relatively low potency against the vasodepressor response of either acetylcholine or McN-A-343, a greater effect was observed in the vasopressor and sialagogic responses of McN-A-343. It is possible that imipramine and methylphenidate block the pressor response of McN-A-343 by inhibiting the release of norepinephrine from the sympathetic nerve ending since a high dose of imipramine was also reported to block the pressor response of dimethylphenylpiperazinium (DMPP) (1). However, the blockade of pressor response from McN-A-343 parallels the antisialagogic effect of imipramine and methylphenidate. This latter effect of McN-A-343 has been shown to be independent of the sympathetic ganglia and the adrenal medulla (8). The antisialagogic test with McN-A-343 is also a more selective test for anticholinergic activity since it is not blocked by cocaine which antagonizes the pressor response of McN-A-343 and tyramine.

It is noteworthy that atropine did not show the selective antagonism among the muscarinic responses as did imipramine and methylphenidate. This difference serves to illustrate the importance of selecting the animals test system which is most pertinent in the clinical use of drugs.

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Determination of Total Steroid Bases in *Solanum* Species

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Abstract □ A method is described whereby relatively small samples of leaves or berries may be screened for their alkaloid content. The glycoside is extracted by the usual methods and the partially pure product hydrolyzed to the aglycone. The aglycone is complexed with methyl orange and the colored complex extracted into chloroform and determined colorimetrically using solasodine as a standard. The colored complex obeys Beer's law in concentration from 10–120 mcg. in 5 ml. chloroform and has its maximum absorption at 420 mμ. The identity of the individual bases present may be determined by other methods such as chromatography.

Keyphrases □ Solasodine determination—*Solanum sodomaeum* and *S. laciniatum* □ Colorimetric analysis—spectrophotometer □ Methyl orange—color reagent

The steroid bases of the solasodine group occur naturally as the glycoside usually containing three sugars. On hydrolysis the glycosides yield the steroid alkaloid in the aglycone form. For example, the glycoside solanine yields solasodine and glucose, galactose, and rhamnose.

Alkaloid content of this material is usually determined by extraction of the dried material using continuous extraction apparatus, removal of the solvent, and precipitation of the bases by ammonia, followed by dissolving in acid, reprecipitation, drying, and weighing of the crude base. The resultant product is still impure and needs further purification by crystallization from an EtOH–water mixture. As some alkaloid is left in the mother liquor, this involves some losses and the determination is consequently inaccurate.

From a literature review of analytical methods for the determination of these bases the following methods have been noted.

Ruzhentseva and Tubina (1) use 4-hr. extraction by 5% acetic acid and precipitation by NH_4OH, 2-hr. drying and a further 2-hr. extraction by MeOH, and final potentiometric titration with 0.1 *N* HCl.

Wierzchowski (2) applies extraction by dilute acetic acid, precipitation by ammonia, solution in EtOH, and color formation using antimony chloride in concentrated HCl.

Balcar and Zalecka (3) use acetic acid extraction, hydrolysis, neutralization, and complex formation with bromothymol blue at pH 8.0 followed by colorimetric estimation.

It was observed that solasodine forms, with methyl orange, a yellow-colored complex which is soluble in chloroform, but at the same time the unhydrolyzed glycoside is not complexed. This was investigated further and it was established that the intensity of

the colored complex is directly proportional to its concentration, and obeys Beer's law over the range from 10–120 mcg. in 5 ml. of solvent.

A method has been developed which permits estimation of the amount of solasodine in fresh berries of *Solanum sodomaeum*, in leaves and berries of *S. laciniatum*, and also in some dried products of these materials.

EXPERIMENTAL

Reagents—*Standard Solution of Solasodine*—Weigh out exactly 10 mg. of pure solasodine and dissolve in 25 ml. of 20% acetic acid A.R. Dilute an aliquot a further 10 times with 20% acetic acid. This solution contains 40 mcg./ml.

Acetate Buffer pH 4.7—Dissolve 5.44 g. sodium acetate A.R. in water; add 2.40 ml. of acetic acid and adjust volume to 100 ml. with water.

Methyl Orange—0.05% solution in water.

Chloroform A.R.

Procedure—*A. Preparation of Standard Curve Using Solasodine as Reference*—Into four suitable separators are pipeted 0, 1, 2, and 3 ml. of 40 mcg./ml. standard solution, and the volume of each is made up to 5 ml. with 20% acetic acid. To each separator 5 ml. of acetate buffer and 1 ml. of methyl orange are added. After shaking for 10 sec., 5 ml. of A.R. chloroform is added. The separators are stoppered and shaken for 3 min. After standing for a few minutes the chloroform layers are withdrawn into dry test tubes, dried with a small amount of anhydrous Na_2SO_4, and absorbances read on a spectrophotometer at 420 mμ using 10-mm. cells. From the readings a standard curve is constructed.

B. Determinations on Dry Leaves and Fruit of Solanum sodomaeum—One-hundred milligrams of finely powdered material and 40 ml. of 95% EtOH are refluxed in a 100-ml. flask for 30 min. The extract is then filtered off using a small Hirsh funnel. The residue on the filter is washed twice with 2 ml. of EtOH, the washings are added to the original filtrate, and transferred into a 50-ml. standard flask, the volume being adjusted to the mark with 95% EtOH. Five milliliters of this solution is pipeted into a 25.4 × 1.9-cm. test tube and EtOH completely removed by evaporation on a water bath while gently blowing an air current into the tube.

The residue is treated with 3 ml. of 1 *N* HCl and hydrolyzed for 2 hr. on a 100° water bath, using as condensers "cold fingers" (elongated test tubes, filled with cold water and inserted in main tubes).

The acid is neutralized by adding 3 ml. of 1 *N* NaOH. Two milliliters of concentrated acetic acid is then added and the contents transferred to a 10-ml. standard flask, the volume being adjusted to the mark with water. One milliliter of this solution is equivalent to 1 mg. of dry material. One to three milliliters is then pipeted into a separator and the procedure followed as for *A*.

C. Determinations on Fresh Berries of Solanum sodomaeum—One-hundred grams of fresh berries is homogenized with 100 ml. of 2% acetic acid in a suitable mincer or blender,[1] to produce a fine pulp which is further diluted with 400 ml. of 2% acetic acid, transferred into two 500-ml. conical flasks, and shaken for 3 hr. The volume is measured and the suspension centrifuged. From the supernatant an amount equal to one-tenth of the measured volume is transferred into a 150-ml. beaker, heated till boiling, and the alkaloid precipitated by addition of 1:2 ammonia in water until the pH reaches 9.5–10.0. The content is transferred into a 100-ml. conical centrifuge tube and spun for 15 min. at 2,000 r.p.m. The supernatant is removed by suction or decanting and the precipitate dissolved in 1 *N* HCl. The solution is transferred to a 100-ml. volumetric flask and adjusted to the mark with 1 *N* HCl. It is then filtered through paper into a dry vessel and 5 ml. pipeted into a small flask for hydrolysis on a 100° water bath by refluxing for 2 hr.

[1] Waring blender.

Table I—Alkaloids in *Solanum sodomaeum* Determined as Solasodine

Solasodine Found	Pure Solasodine Added, mcg.[a]	Plus Pure Solasodine[a] Calcd., mcg.	Found, mcg.	% Recovery
In Dried Berries, mcg./mg.				
56	20	76	78	102.6
56	40	96	101	105.2
57	20	77	72	93.5
57	40	97	99	102.1
In Fresh Berries, mcg./5 mg.				
65	71	136	134	98.5
63	70	133	132	99.2

[a] Added as unhydrolyzed solanine.

To the flask is then added 5 ml. of 1 *N* NaOH and 20 ml. of concentrated acetic acid, the contents transferred into a 100-ml. volumetric flask, and adjusted to the mark with water. Each milliliter of this solution is equivalent to 5 mg. of fresh berries.

One to three milliliters of this solution is transferred into a separator and the procedure followed as for *A*.

Evaluation of Method—The method was evaluated by assaying samples of dried and fresh berries together with similar samples to which known amounts of the unhydrolyzed glycoside solanine were added. It is known that solanine contains 46.7% (w/w) solasodine, and the theoretical expected result could thus be readily calculated. All samples were subjected to the same procedure of extraction, hydrolysis, and color complexing as previously described. The experimental results are shown in Table I.

DISCUSSION

The method developed for determination of solasodine is also applicable to similar nitrogen-containing alkaloids, such as tomatine. The colored complex with methyl orange is formed only by an aglycone after hydrolysis. The glycosides do not form such complexes. The assay result is expressed in terms of a chosen standard, in this case solasodine. Colored complexes of solasodine are also formed with bromocresol purple and thymol blue, giving yellow complexes extractable by chloroform at pH 3–4. The assay is conducted as described and for formation of the complex with methyl orange, the sample is always dissolved in 20% acetic acid. When conditions for formation of the complex result in acetic acid concentrations varying from those described, the standards must also be prepared in exactly similar acetic acid concentration and have the same pH value. Several samples of fresh and dried products and also several samples obtained during processing of berries were assayed by this method and found to be satisfactory.

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