In an attempt to delineate the mechanism of action of this compound a number of pharmacologic techniques were employed. Atropine sulfate, 0.5 mg./Kg., did not alter the action of the compound. It was subsequently determined that the compound could effectively block the following procedures: electrical stimulation of the peripheral end of the right vagus, the carotid occlusion pressor reflex, and the pressor effect of nicotine salicylate. In addition, potentiation of the pressor response to epinephrine was noted as well as elimination of the reflex vagal effect that usually develops at the height of the epinephrine response. These findings were observed in 3 animals 20 to 30 min. following administration of the compound.

CONCLUSIONS

The evidence points to ganglionic blockade as the principal mechanism of action of this agent, however, the possibility that it may also exert some direct depressant action on the cardiovascular system has not been eliminated.

SUMMARY

Five new derivatives of quinuclidine have been prepared by reacting 3-quinuclidinol with various aminoalkyl halides *via* the Williamson synthesis. The resulting ethers, as the dimethiodide salts, were tested for antihypertensive activity. All showed varying degrees of activity, the most effective being 3-(3-dimethylaminopropoxy) quinuclidine.

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Determination of Moisture in Crude Drugs by Gas–Liquid Chromatography

By NOURI Y. MARY

The use of gas–liquid chromatography was investigated as a means of determining the moisture content of crude drugs. Sixteen crude drugs representing a variety of plant parts and products were analyzed using this technique. Water was extracted from the crude drugs by disintegration with anhydrous methanol in a Waring blender and analyzed with a column of Teflon-6 coated with 10 percent polyethylene glycol 1500, using *n*-propanol as an internal standard. Peak height ratios were used to calculate the moisture content. The results obtained with the gas–liquid chromatographic procedure were quantitatively in accord with the results obtained by oven drying at 105° and toluene distillation methods. In addition, the new chromatographic procedure is simpler, more rapid, and has a very good precision (the maximum standard deviation was 0.36).

THE USE of gas–liquid chromatography for the direct analysis of moisture in a number of materials has been reported by many investigators. This technique was found to be reliable and convenient. Most of these studies utilized the method for the analysis of aqueous solutions of organic compounds (1–8). In only a few instances has this method been applied to the determination of water in natural products. The National Bureau of Standards (9) has adopted gas–liquid chromatography for moisture determination of grains. Schwecke and Nelson (10) used gas–liquid chromatography in determining the moisture content of cereal pellets, dried raisins, flour, and other food, and Brekke and Conrad (11) measured the water content of fruits and fruit products employing the same technique.

The simplicity, rapidity, and accuracy of the gas chromatographic process should also make the method well suited to the estimation of moisture in crude drugs. The methods currently recognized in the USP and NF for moisture content measurement of crude drugs include oven drying and toluene distillation. Both of these methods are time-consuming, and in the case of toluene distillation, the use of a comparatively large sample of the crude drug is a further disadvantage. The present investigation was, therefore, undertaken in order to develop an alternate procedure for the rapid measurement of moisture content in crude drugs utilizing a direct gas–liquid chromatographic technique.

EXPERIMENTAL

The crude drugs used in this work were obtained from S. B. Penick and Co., New York, N.Y. All drugs, except digitalis and peppermint leaves, were in powdered form. The leaf samples of digitalis and peppermint were powdered, before analysis, according to the NF XII (12) specification.

Moisture Determination by Oven Drying—The moisture content of all of the crude drugs, except

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cinnamon, clove, ginger, and peppermint was determined by the NF XII method (12) for drugs containing no constituents volatile at 105°. The moisture content of cinnamon, clove, ginger, and peppermint was determined by the NF XII method (12) for drugs containing ether-soluble constituents volatile at 105°.

Moisture Method by Toluene Distillation—The azeotropic procedure described in the USP XVII (13) was followed in determining the moisture content of all of the crude drugs.

Moisture Determination by Gas–Liquid Chromatography—*Apparatus*—The chromatograph used in this study was a Perkin-Elmer model 154 vapor fractometer equipped with a dual chamber thermistor thermal conductivity cell.

Column Packing—The column used was a stainless steel tubing, 2 m. long and 0.625 cm. in o.d. packed with Teflon-6, 35-mesh, coated with 10% polyethylene glycol (PEG) 1500.[1] The packed column was purchased from Perkin-Elmer, Norwalk, Conn., and was conditioned at 100° for 24 hr. before use.

Chromatography Conditions—The operating temperature was 100°, the detector voltage was 8 v., and the helium carrier gas flow rate was 65 ml./min. The sample size injected was 4 μl.

Standard Curve—The internal standard used in this work was *n*-propanol, which is eluted before the water. Elvidge and Proctor (2) used *n*-propanol and obtained good separation from water in their analysis of aqueous pharmaceutical preparations.

A standard curve was prepared by chromatographing samples containing various amounts of water in mixtures of 3 ml. *n*-propanol and 100 ml. anhydrous methanol. The ratio of peak height of water to *n*-propanol was calculated from the chromatogram and plotted against the corresponding weight[2] ratio of the two compounds; a linear relation was obtained (Fig. 1). Although the standard curve appeared to be very reproducible (the standard deviation of peak height ratios for the standard curve noted at four different runs was 0.047), new standard solutions were prepared every day that unknown samples were chromatographed.

Calculations—Samples of unknowns were chromatographed under the same conditions as the standards. The amount of moisture in the unknown was determined by computing the water–*n*-propanol peak height ratio from the chromatogram, obtaining the corresponding weight ratio from the standard curve, and multiplying by the weight of *n*-propanol. The value so obtained was then converted to percent moisture in the original drug sample.

Extraction Procedure—The procedure used to remove water from the crude drugs was a simple disintegration of the drug with anhydrous methanol in a Waring blender. This procedure has been utilized, with good quantitative results, in the extraction of water from a variety of food products (10, 11).

A 15-Gm. sample of the crude drug to be analyzed was transferred to a stainless steel Waring blender fitted with air-tight screw cap and threaded sampling plug. One hundred milliliters of anhydrous methanol and 3.00 ml. of *n*-propanol were added and

[1] Carbowax 1500, Union Carbide Corp., New York, N.Y.
[2] Water and *n*-propanol were measured by volume and converted to weight using specific gravity calculations.

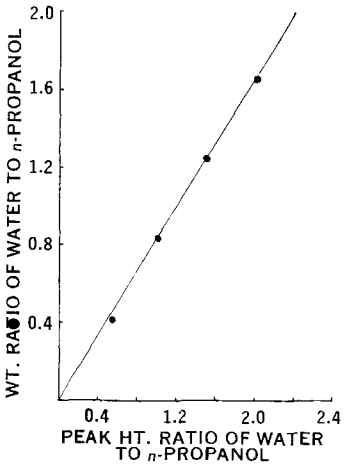

Fig. 1—Weight/peak height ratios relation of water to n-propanol.

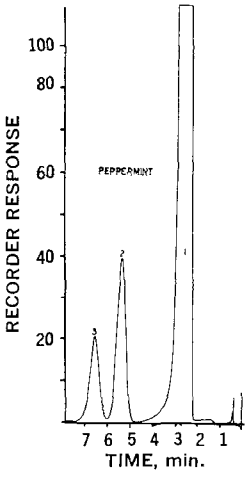

Fig. 2—Typical gas chromatogram of the methanol extract from crude drug (peppermint.) Key: 1, methanol; 2, n-propanol; 3, water.

the sample was disintegrated for 5 min. After blending, the mixture was allowed to settle for 1 min., a sample of the supernatant was transferred with a pipet into a 10-ml. vial, and 4 μl. was injected with a Hamilton microliter syringe into the gas chromatograph.

RESULTS AND DISCUSSION

Water exhibits tailing during elution, particularly on nonpolar liquid phases or on those of intermediate polarity, and on solid supports with appreciable adsorptive properties (14). The separation and quantitative analysis of water has been markedly improved by the use of highly polar liquid phases and inert supports of fluorinated hydrocarbon polymers. Recent work on the analysis of various aqueous systems (5, 8, 10, 11) has shown that the use of columns of Teflon powder coated with 5–20% PEG produced water peaks with greatly reduced tailing and low retention time. The chromatographic column used in this work was Teflon-6 powder coated with 10% PEG 1500. It afforded good resolution and symmetrical peaks. In addition, the retention time of water was low; this allowed its rapid analysis in the extracts. Peak heights were used in the calculation, since standard operating conditions could be well reproduced,

TABLE I—PERCENT MOISTURE IN CRUDE DRUGS AS DETERMINED BY OVEN DRYING (OD), TOLUENE DISTILLATION (TD), AND GAS–LIQUID CHROMATOGRAPHY (GLC) METHODS

Crude Drug	OD[a]	TD[a]	GLC[b]
Agar	15.13	14.00	14.72
Aloe (Cape)	5.00	4.00	5.47
Belladonna root	6.66	6.32	6.43
Cascara sagrada	7.09	6.32	7.11
Colchicum corm	6.22	6.63	5.74
Powdered digitalis (*D. purpurea*)	4.21	4.00	3.89
D. purpurea leaf	5.80	5.49	5.92
D. lanata leaf	7.01	6.00	7.07
Ergot	5.64	5.03	5.55
Gentian	6.07	5.99	6.08
Glycyrrhiza	7.49	6.32	6.87
Starch (corn)	8.69	8.66	9.00
Cinnamon	6.56	6.65	7.38
Clove	5.42	5.44	4.60
Ginger	6.84	6.50	5.95
Peppermint	7.12	6.74	6.91

[a] The values are the average of two determinations each. [b] The results are the means of five chromatographic injections, representing two or more extractions of each crude drug sample.

TABLE II—REPRODUCIBILITY OF GLC PROCEDURE[a]

	% Moisture	
	3-min. Blending	5-min. Blending
Cascara sagrada	6.91, 6.99	6.99, 7.23
D. purpurea leaf	5.78, 5.95	5.78, 6.08
Ergot	5.47, 5.79	5.47, 5.63
Gentian	5.95, 6.11	6.08, 6.08
Starch (corn)	9.08, 9.32	8.92, 9.08

[a] Each value recorded represents the mean of five chromatographic injections from an extract of the crude drug.

the peaks were sharp and symmetrical, and the half-peak widths of water and the standard were essentially equal. A typical chromatogram of the methanol extract from peppermint appears in Fig. 2.

The moisture content of the crude drugs analyzed by gas–liquid chromatography are given in Table I. The results are compared with those obtained by oven drying at 105° and toluene distillation methods. The values obtained with the gas–liquid chromatographic procedure in each case are quantitatively in accord with the values given by the other two methods. The reproducibility of the chromatographic analysis is satisfactory. The maximum standard deviation found was 0.36. The reproducibility of the method for five crude drugs can be readily seen from an examination of the figures shown in Table II.

In preparing the extracts for chromatographic analysis, the crude drugs were disintegrated with anhydrous methanol in a Waring blender for 5 min. Although it seemed that 3 min. of blending were adequate (Table II), the drugs were disintegrated for 5 min. to insure the complete extraction of their water content. The results show that the increased blending time did not increase the values for moisture content. While the gas–liquid chromatographic method furnishes a reliable value for water extractable by methanol, there is a good reason to believe that this water consists of all but that which is chemically combined with the sample of the crude drug (9). This chemically combined water represents a negligible amount of the total moisture present in the drug.

It was possible in this work to evaluate some of the technical problems encountered with each of the three methods. Where it is certain that the loss on drying of a drug is actually water, moisture determination by oven drying is a fairly quantitative procedure. Since a large number of vegetable drugs contain variable amounts of volatile substances other than water, oven drying is not generally applicable except for rough estimations, and the toluene method is to be preferred. However, some disadvantages are also inherent in the toluene distillation method. Moisture determination by this method requires considerable care and attention during operation, particularly when frothing or similar difficulties occur; previous cleaning of the condenser and collecting tubes with dichromate–sulfuric acid solutions does not always prevent the collection of droplets of water on the internal surface, particularly if the material contains traces of volatile fatty acids. Moreover, large amounts of a drug, in some cases more than 50 Gm., must be used, and the direct use of a solvent like toluene without dispersion of the material may fail to obtain the release of all the moisture from the crude drug, a fact which was especially noticeable in this work in the case of aloe. Gas–liquid chromatography, on the other hand, is simple, requires a relatively small sample size, has very good precision, and is more selective than oven drying or toluene distillation methods. When the speed of the three methods is compared, it is found that the chromatographic method is much more rapid. Once the standard curve has been prepared, a determination by gas–liquid chromatography can be completed in a maximum of 15–20 min. Oven drying requires more than 5 hr. for drugs containing no volatile constituents and more than 48 hr. for drugs containing ether-soluble constituents which volatilize at the oven temperature used, while a determination by toluene distillation takes from 3–4 hr.

When all these factors are considered, it can be readily seen that the new chromatographic method is distinctly superior. It surpasses oven drying and toluene distillation in many ways and should be easily adaptable to moisture estimation in a variety of crude drugs and similar natural products.

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