THE OCCURRENCE OF METHYL COMPOUNDS IN GALENICALS

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In a recent paper in this journal Brookes and Johnson[1] described an investigation into the occurrence of methyl compounds in certain galenical preparations. This is a subject of considerable importance to the Department of the Government Chemist and to all pharmaceutical houses that prepare galenicals containing spirit, since the presence of methyl alcohol is often regarded as *prima facie* evidence of the presence of Industrial Methylated Spirits. The suggestion was made in the paper and also in the ensuing discussion that in certain cases insufficient allowance was made for naturally occurring methyl alcohol, or methyl compounds which could give rise to methyl alcohol in the course of analysis.

In particular, the case of senega was examined in some detail, and it appeared that the addition of acid in the distillation of the root or its preparations could increase considerably the yield of methyl alcohol, presumably by hydrolysis of methyl compounds present in the material. This was significant, as acid distillation, with the object of "fixing" free ammonia, is prescribed in the British Pharmacopœia for the estimation of spirit in senega tinctures, and the expectation that this procedure would give a higher yield of methyl alcohol than that arising from distillation with no addition of acid was given experimental support by the authors. In this connection, it may be noted that the Government Laboratory method for senega tinctures does not follow the B.P. procedure in this respect, but, instead, employs a preliminary distillation with no addition of acid followed by acidification and redistillation of the distillate, so that our practice is here in accordance with that recommended by Brookes and Johnson. At our request, a number of samples connected with the above investigation, including some of the original materials, were kindly made available to the Government Laboratory, and the results of some independent experiments are given in the present paper.

Two methods of estimation of methyl alcohol were employed: (a) the modified Denigès test as described in the B.P. (p. 49), applied quantitatively by matching with standards, and (b) as a check on this method, one based on the colour reaction of chromotropic acid with formaldehyde as described at the end of this paper. It will be seen from Tables II and III that very considerable differences in the estimated methyl alcohol content of distillates are given in certain cases by the two methods, and that sometimes the Denigès test was found to give a definite positive reaction when the chromotropic acid test showed no appreciable reaction. As a precaution against the possible inhibition of the chromotropic acid colour reaction in such circumstances, repeat determinations were made

after making small measured additions of methyl alcohol to test-portions of the distillates, and the normal development of the colour was observed. Chromotropic acid appears, therefore, to be a more specific reagent for formaldehyde than is Schiff's reagent, and there seems to be no doubt that, in the case of relatively impure distillates, the Denigès test can be misleading. As Brookes and Johnson used the modified Denigès test exclusively, this is of great importance in its bearing on the validity of some of their experimental figures.

For example, while the figures obtained here on samples 1 to 5 (concentrated compound infusion of gentian—see Table I) show fair agreement with those given by Brookes and Johnson, both by the Denigès and

TABLE I

Concentrated Compound Infusion of Gentian	Methyl Alcohol per cent. v/v in Galenical		
	By Denigès test	By Chromotropic acid test	Brookes and Johnson
(1) No. 1 ...	0·06	0·08	0·05
(2) No. 2 ...	Trace	Trace	0·025
(3) No. 3 ...	0·02	0·05	0·05
(4) No. 4 ...	0·02	0·04	0·05
(5) No. 5 ...	0·02	0·04	0·05

chromotropic acid tests, this is not the case with sample No. 6 (concentrated infusion of senega). In Table II it will be seen that experiments 2, 3, 4, 5 and 8 show very different results as between the Denigès and chromotropic acid methods. The apparently large yields of methyl alcohol obtained by acid distillation, and from the redistillation of residues with acid, which are indicated by the former method are not con-

TABLE II

Sample No. 6 Concentrated Infusion of Senega	Methyl Alcohol per cent. v/v in Galenical	
	By Denigès Test	By Chromotropic Test
(1) Distillation with no addition of acid	0·12	0·13
(2) Residue from above redistilled after adding water, alcohol and 2 ml. of concentrated sulphuric acid	0·19	Trace
(3) Above residue again distilled after adding water and alcohol ...	0·14	Trace
(4) Acid distillation	0·36	0·16
(5) Residue from above redistilled after adding water and alcohol	0·19	Trace
(6) Distillation with ammonia (5 ml. of concentrated solution) the distillate being acidified and redistilled	0·15	0·16
(7) D acid D (G.L. method—see text)	0·12	0·12
8) Residue from above redistilled after adding water, alcohol and 3 ml. of concentrated suphuric acid	0·19	Trace

firmed by the latter. This is again demonstrated in Table III, and the marked differences may be attributable mainly or entirely to interfering volatile substances that react with Schiff's reagent under the conditions of the Denigès test; the findings of Brookes and Johnson in connection with liquid extract of senega need re-examination in this light. Thus, they reported in Table III of their paper 1·40 per cent. of methyl alcohol

(Denigès) by acid distillation in a sample of the liquid extract and only 0·01 per cent. by distillation with no addition of acid. Further, successive acid distillations of the residue left after plain distillation were reported to yield a total of 1·36 per cent. of methyl alcohol (Denigès).

TABLE III

Senega Root	Methyl Alcohol per cent. w/w in Root	
	By Denigès Test	By Chromotropic Acid Test
(1) 20 g. of root distilled with water and alcohol	Trace	Trace
(2) Residue from above redistilled after adding water, alcohol and 3 ml. of concentrated sulphuric acid	1·12	0·50
(3) 20 g. of root distilled with water, alcohol, and 3 ml. of concentrated sulphuric acid	0·95	0·42
(4) Residue from above redistilled after adding water and alcohol	0·55	0·37
(5) The same residue again redistilled after adding water and alcohol	0·30	0·15
(6) The last operation repeated	0·19	0·04
Total	1·99	0·98
(7) 20 g. of root distilled with water, alcohol and 5 ml. of concentrated solution of ammonia, the distillate being acidified and redistilled	0·50	0·50

In view of our findings given here in Table II, these figures are clearly in need of verification, and this applies also to the figures of 0·36 per cent. of methyl alcohol (Denigès) reported by Brookes and Johnson as arising from acid distillation of a sample of concentrated infusion of senega against 0·04 per cent. by distillation with no addition of acid. It is suggested that, had the more specific chromotropic acid test been applied here, much lower figures for methyl alcohol by acid distillation would have been obtained. In this connection it is interesting to observe (cf. Table II expt. 6 and Table III expt. 7) that with distillation in the presence of excess of ammonia, the two methods give practically identical results. As might be expected, our routine procedure (distillation, acidification, redistillation) gives substantially the same figure by either test (see Table II—7) and agrees with that found by distillation with no addition of acid (see Table II—1).

Repeated acid distillation of senega root (Table III) yielded a total of about 1 per cent. w/w of methyl alcohol as indicated by the chromotropic acid test (or apparently about 2 per cent. by the Denigès test), and it was confirmed that ammonia acts similarly to acid in increasing the yield of methyl alcohol (7). However, there is no reason to believe that more than a small fraction of this potentially large amount of methyl alcohol could appear in the galenicals as prepared by the process of extraction prescribed in the B.P., especially if acid hydrolysis is avoided in the analysis.

Thus, Brookes and Johnson found, by distillation with no addition of acid, 0·04 per cent. and 0·09 per cent. of methyl alcohol in samples of the infusion (a 40 per cent. w/w preparation) which, it is stated, were

prepared by a modification of the B.P. method involving the presence of ammonia during the percolation and, therefore, the possibility of some generation of methyl alcohol by alkaline hydrolysis. Further, distillation with no addition of acid yielded only 0·01 per cent. of methyl alcohol from a sample of the liquid extract (which is a 1 : 1 preparation).

Summary

(1) Methyl alcohol or substances that can give rise to methyl alcohol can occur as natural constituents in certain galenicals, and only where amounts of methyl alcohol are detected in excess of normal limits should they be regarded as evidence of the presence of Industrial Methylated Spirits. This has long been a guiding principle at the Government Laboratory.

(2) It is recognised that, in the case of senega preparations, a preliminary distillation with no addition of acid should precede the methyl alcohol test. This is recommended by Brookes and Johnson; it is preferable to the acid distillation prescribed in the B.P., and is in accordance with Government Laboratory practice.

(3) Some of the findings of Brookes and Johnson concerning the extraction of relatively large amounts of methyl alcohol from senega preparations have been shown to be of doubtful validity owing to the lack of specificity of the Denigès test.

(4) From their figures obtained by distillation with no addition of acid, Brookes and Johnson have not shown that concentrated infusion of senega can have a methyl alcohol content of more than about 0·1 per cent., and they found only 0·01 per cent by this method in a sample of the liquid extract.

Over many years we have examined many samples of concentrated infusion of senega by our routine method; a fair proportion of them indicate complete absence or only a trace of methyl alcohol. It seems probable that slight variations exist between different batches of senega root due to age, origin or variety, and that these variations may give rise to senega preparations containing varying but very small amounts of methyl alcohol.

Description of Chromotropic Acid Method

This procedure was developed for the special purposes required for this investigation and is based on a method described by Boos[2].

To 1 ml. of the test-solution (containing 1 per cent. v/v of ethyl alcohol and not more than 0·1 mg. of methyl alcohol) contained in a 5 × $\frac{3}{4}$″ test-tube, add 8 drops of Denigès permanganate solution (3 g. of potassium permanganate and 15 ml. of 85 per cent. phosphoric acid per 100 ml. of solution) and shake to mix. After standing for 10 minutes at room temperature, add sufficient freshly-prepared saturated sodium bisulphite solution drop by drop (about 2 drops) to reduce excess of permanganate, and transfer the tube to an ice-bath. Cool for about a minute and pour 4 ml. of concentrated sulphuric acid gently down the

side of the tube. Cool for a further 2 minutes and then shake to mix, continuing the cooling for a short time. Add 1 drop of 2 per cent. chromotropic acid solution, shake to mix, and heat in a water-bath for 15 minutes at 60°C. Transfer the tube to the ice-bath and, after cooling, dilute the solution with 3 or 4 ml. of water, cool and rinse into a Nessler tube to a total volume of about 15 ml. Compare the colour of the solution, viewed vertically against a white background, with that of standards similarly prepared from solutions containing 0 to 0·1 mg. of methyl alcohol in 1 per cent. ethyl alcohol.

Chromotropic acid reagent. Dissolve 0·1 g. of chromotropic acid in 5 ml. of cold water and filter the solution. This solution darkens on standing and is usable for a few days only, after which, matching of the violet reaction colour becomes difficult. For this reason, although the violet colour is stable, fresh standards should be prepared on the same day as the test.

Sensitivity. 0·01 mg. of methyl alcohol is readily detected in 1 ml. of test solution. The violet colour increases in depth for amounts of methyl alcohol up to about 0·5 mg., but beyond this approximate limit there is a diminution in intensity.

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REFERENCES

1. Brookes and Johnson, *J. Pharm. Pharmacol.*, 1949, **1**, 777.
2. Boos, *Anal. Chem.*, 1948, **20**, 964.