RESEARCH PAPERS

THE STABILITY OF RESCINNAMINE IN SOLUTION

From The Joint Committee of The Pharmaceutical Society of Great Britain and The Society for Analytical Chemistry on Methods of Assay of Crude Drugs

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The ultra-violet absorption characteristics of solutions of rescinnamine in various solvents change when the solutions are exposed to daylight. The change, which is thought to be due to a *cis-trans* isomerism, is not accompanied by any detectable alteration in pharmacological properties.

IN 1956 the Pharmaceutical Society of Great Britain and the Society for Analytical Chemistry formed a Joint Committee to investigate methods of assay of crude drugs. A Working Panel* was set up, under the direction of the Committee, to examine methods of assay for rauwolfia.

While examining a method of assay for rauwolfia described by Carol, Banes, Wolff, and Fallscheer[1] variable results were obtained in some cases. The assay method, which was claimed to give a measure of both the reserpine and rescinnamine contents of rauwolfia, depends upon separation of these two weak bases from others present, followed by their hydrolysis to trimethoxybenzoic and trimethoxycinnamic acid respectively. The two acids are then determined in solution in chloroform by an ultra-violet absorption method making use of a two-point correction technique.

TABLE I

THE STABILITY OF TRIMETHOXYCINNAMIC ACID IN BORATE BUFFER SOLUTION (PH 10) WHEN STORED EXPOSED TO DAYLIGHT

Time after preparation of solution	*E* (1 per cent, 1 cm.) values at 290 mμ			
	Laboratory			
	A	B	C	D
Immediately	776	810	784	798
10 minutes	772	788	762	436
20 minutes	772	774	756	292
30 minutes	772	756	731	288
2 hours	718	560	589	288
24 hours	384	286	265	290
24 hours (in dark) ..	796	808	788	802

Samples of pure trimethoxybenzoic and trimethoxycinnamic acids were prepared and their light absorption characteristics in various solvents determined. Although good agreement could be obtained between four laboratories for the trimethoxybenzoic acid there was a wide discrepancy

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in results for the trimethoxycinnamic acid. Further tests showed that although solutions of the latter acid are stable for several hours when protected from light the ultra-violet absorption characteristics of solutions exposed to daylight change rapidly. Solutions were prepared in various solvents and exposed to daylight, and the extinction of 1-cm. layers were measured at intervals during 24 hours. The figures recorded in Table I show that although the rate of change was not the same in the various laboratories, the trend was similar in each case. In solvents such as borate buffer solution (pH 10) and methanol the wavelength of maximum absorption changed from about 290 mμ to about 270 mμ and the extinction of a 1-cm. layer of an 0·0005 per cent w/v solution at 290 mμ fell from about 0·400 to 0·145 within 24 hours or a little more. Further exposure to daylight brought about no further change. In chloroform, the solvent used by Carol and others, the maximum absorption of a fresh solution occurred at about 310 mμ and on exposure to light the extinction value decreased markedly although the wavelength of maximum absorption did not change very much. Figure 1 shows the absorption curves of a 0·0005 per cent solution of trimethoxycinnamic acid in chloroform before and after exposure to daylight.

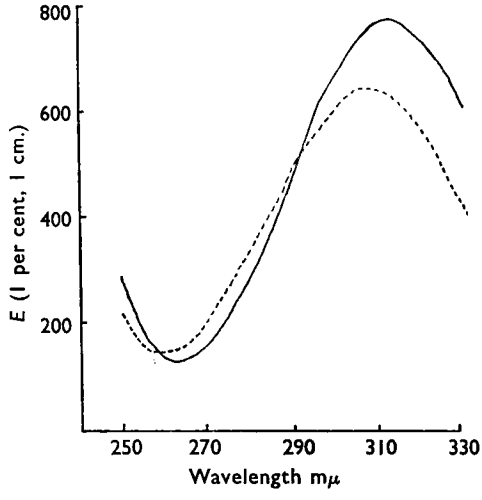

FIG. 1. Absorption curves of trimethoxycinnamic acid (0·0005 per cent) in chloroform
——— Before exposure to light.
- - - - - After 24 hours' exposure to light.

Because of these findings the light absorption characteristics of reserpine and rescinnamine were examined in a similar manner. Those of reserpine

TABLE II

THE STABILITY OF RESCINNAMINE IN ACETIC ACID SOLUTION WHEN STORED EXPOSED TO DAYLIGHT

Time after preparation of solution	*E* (1 per cent, 1 cm.) values at 300 mμ			
	Laboratory			
	A	B	C	D
Immediately	411	400	411	435
10 minutes	411	357	363	358
20 minutes	405	332	339	340
30 minutes	397	325	333	336
2 hours	356	315	322	332
24 hours	322	315	322	335
24 hours (in dark)	411	400	411	435

solutions showed little alteration when exposed to light for several hours, but those of rescinnamine solutions showed a marked and rapid change. Tables II,III and IV show the values of the extinction coefficients recorded for approximately 0·002 per cent w/v solutions of rescinnamine in 10 per

TABLE III

THE STABILITY OF RESCINNAMINE IN CHLOROFORM WHEN STORED EXPOSED TO DAYLIGHT

Time after preparation of solution	*E* (1 per cent, 1 cm.) values at 306 mμ Laboratory			
	A	B	C	D
Immediately	393	385	—	394
10 minutes	375	355	—	320
30 minutes	351	332	—	312
2 hours	317	305	—	300
24 hours	295	293	—	296
24 hours (in dark) ..	394	385	—	407

cent acetic acid, chloroform and methanol respectively, while Figure 2 shows light absorption curves of a solution in acetic acid before and after exposure to daylight for 24 hours. The discrepancy in the initial extinction values obtained in the different laboratories might be due to changes already taking place during preparation of the solutions. About 24 hours exposure to daylight brings about the changes after which further exposure has no effect.

Two possible reasons for the changes have been considered and these are, (i) splitting of the double bond, and (ii) *cis-trans* isomerism.

The light absorption curve of a solution of trimethoxycinnamic acid in borate buffer solution is much more like that of a solution of trimethoxybenzoic acid after irradiation than it was originally. The relationship between the three curves is shown in Figure 3. This seems to support the first of the two possibilities but on the whole the second seems the more likely explanation. Changes in configuration can occur in substances capable of existing in geometrically isomeric forms when their solutions are exposed to light. For example,

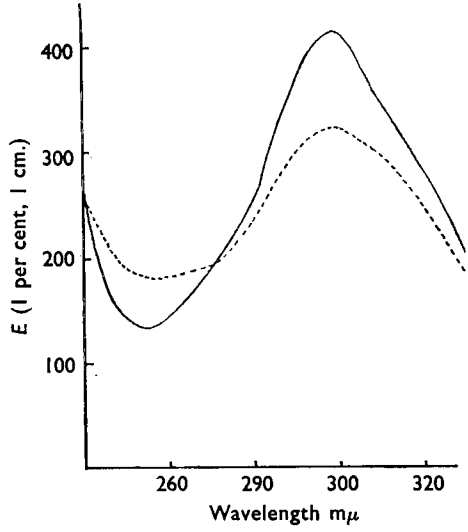

FIG. 2. Absorption curves of rescinnamine (0·002 per cent) in acetic acid

——— Before exposure to light.
- - - - - After 24 hours' exposure to light.

on exposure to ultra-violet light either maleic or fumaric acid is converted into an equilibrium mixture of the two acids. It is also well known that the light absorption characteristics of geometrical isomers may differ considerably.

TABLE IV

THE STABILITY OF RESCINNAMINE IN ABSOLUTE METHANOL WHEN STORED EXPOSED TO DAYLIGHT

Time after preparation of solution	E (1 per cent, 1 cm.) values at 302 mμ			
	Laboratory			
	A	B	C	D
Immediately	426	472	455	—
10 minutes	401	400	455	—
30 minutes	365	372	425	—
2 hours	343	370	412	—
24 hours	342	370	392	—
24 hours (in dark) ..	419	472	455	—

It was of importance to establish whether or not this change affected the pharmacological action of rescinnamine. An aqueous solution with the minimum quantity of dilute acetic acid was prepared to contain 2 mg. of the alkaloid in each millilitre and was divided into two equal portions, one of which was exposed to daylight for several hours while the other was protected from light. Suitable dilutions were examined spectrophotometrically to ensure that the change had occurred; the solutions were also tested by a modification of the colorimetric method of assay proposed by Banes, Wolff, Fallscheer and Carol[2]. This latter method is based upon the indole moiety of the rescinnamine molecule and thus, if the change were solely due to changes in the trimethoxycinnamoyl moiety of the molecule, it would be expected to give a similar result with solutions both protected and exposed to light. This was found to be the case. The main bulk of each of the two solutions was used for pharmacological tests.

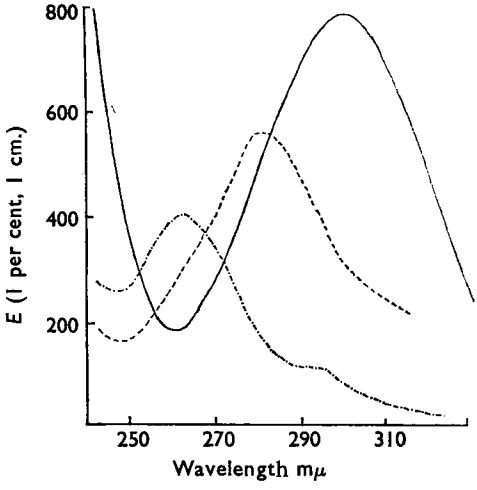

FIG. 3. Absorption curves of trimethoxybenzoic and trimethoxycinnamic acids in borate buffer solution (pH 10)

— · — Trimethoxybenzoic acid.
——— Trimethoxycinnamic acid before exposure to light.
- - - - - Trimethoxycinnamic acid after 24 hours' exposure to light.

The pharmacological tests were carried out by methods similar to those described by Zoha, Kirpekar and Lewis[3]. Briefly, the test solutions were

compared with a control solution on the blood pressure of anaesthetised cats and also upon certain vasopressor reflexes, namely those elicited by stimulation of the central end of the cut vagus, and by compression of the abdominal aorta and of the common carotid arteries. The effect upon the response of blood pressure to stimulation of the splanchnic nerve was also ascertained. No differences could be observed between the control and test solutions.

These qualitative investigations thus indicate that certain pharmacological properties of rescinnamine are not altered by exposing solutions to daylight and the problem therefore resolves itself into a purely analytical one. It is apparent that, without modification, the method of assay of rauwolfia proposed by Carol and others[1] is untrustworthy. It is also clear that the ultra-violet absorption characteristics of rescinnamine cannot be used as a basis for the determination of the alkaloid. It may be that the amount of trimethoxycinnamic acid or of rescinnamine in a solution might be obtained by subjecting the solution to irradiation with ultra-violet light until the light absorption characteristics became constant. Such a procedure could not readily be applied to solutions containing mixtures of reserpine and rescinnamine or of the acids resulting from their hydrolysis, however, since the effect of irradiation is to impair the favourable conditions necessary for the application of the two-point correction procedure.

References

1. Carol, Banes, Wolff and Fallscheer, *J. Amer. pharm. Ass., Sci. Ed.*, 1956, **45**, 200.
2. Banes, Wolff, Fallscheer and Carol, *ibid.*, 1956, **45**, 708.
3. Zoha, Kirpekar and Lewis, *J. Pharm. Pharmacol.*, 1958, **10**, *231T.*