amino acids in the keratogenous zone of hair. Belanger (3) noted localization of methionine-S^{35} and cystine-S^{35} after subcutaneous injections into rats. Fleischer, *et al.* (4), injected S^{35}-yeast protein hydrolysate containing cystine-S^{35}, methionine-S^{35}, and methionine sulfoxide-S^{35} intravenously into rats and found a rapid incorporation of S^{35} into hair, particularly over the length of the intensely vascularized part of the hair. Ryder (5) and Harkness and Bern (6) also found a rapid and marked uptake of S^{35} in the keratogenous zone of anagen follicles after injecting cystine-S^{35}; the latter investigators also noted uptake of C^{14} in the bulb and keratogenous zone after injecting C^{14}-labeled algal protein hydrolysate of undefined amino acid composition into mice.

Bern, *et al.* (7), have pointed out that transport of cystine from the circulatory system *via* absorption through the hair bulb is conceivable, but that a lateral transport from the capillaries around the follicle shaft (8) is also possible.

Apparently, S^{35}-amino acids are at least as equally utilized for hair protein synthesis as for plasma or tissue protein synthesis. The S^{35} specific activity of rabbit hair keratin was found (9) similar to that of the S^{35} activity of tissue proteins following intravenous injections of S^{35}-labeled yeast protein hydrolysate. A much higher specific activity of the cystine-S^{35} in wool than in plasma was noted following the intravenous administration of cystine-S^{35} to sheep (10). The latter concluded cystine is incorporated into wool as a free amino acid.

Only traces of cystine and methionine as free amino acids have been found in aqueous extracts of the roots (bulb, prekeratinized fiber portion, and attached inner root sheath) of keratinized structures, although more methionine is present in the protein of the inner root sheath of hair follicles, as detected by administering S^{35}- and C^{14}-labeled methionine to rats (11) and large quantities of cystine occur in the hair protein itself. According to Rogers (12), the pool of free amino acids in the hair roots is presumably available for keratin synthesis and the small concentration of cystine may be related to a high demand for cystine by the growing fiber and to mechanisms for maximum utilization of the sulfur-containing amino acids. Recently, it has been reported that other amino acids, H^3-labeled alanine and leucine and C^{14}-labeled serine, were incorporated into the growing hair of guinea pigs in the same pattern as cystine-S^{35} after intraperitoneal injection (13); however, no other studies of local utilization following topical application of amino acids by growing hair have been reported.

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Degradation of Phenylephrine Hydrochloride in Tablet Formulations Containing Aspirin

By A. E. TROUP and H. MITCHNER†

The breakdown of aspirin in tablet formulations containing phenylephrine was found to result in a concurrent loss of phenylephrine activity. Specific functional group analysis of the secondary amine function on phenylephrine was necessary to follow degradation in tablet formulations. Analyses with methods based on the phenolic function of phenylephrine did not show similar activity loss. With the use of thin-layer chromatography and comparative chromatograms with synthetic acetylated phenylephrine derivatives, three acetylated phenylephrine degradation products could be identified in tablet formulations. At room temperature the primary degradation pathway was the acetylation of the secondary amine function, but at elevated temperatures, acetylation was found to have progressed to phenylephrine's phenolic and alcoholic groups.

PHENYLEPHRINE HYDROCHLORIDE is frequently incorporated in multi-ingredient pharmaceutical preparations. Analytical assay methods for phenylephrine in such mixtures have been directed both to the development of specific molecular procedures and to methodology involving the separation of the phenylephrine followed by its analysis. Assay problems have been further complicated in the endeavor to evaluate degradation during stability testing where the

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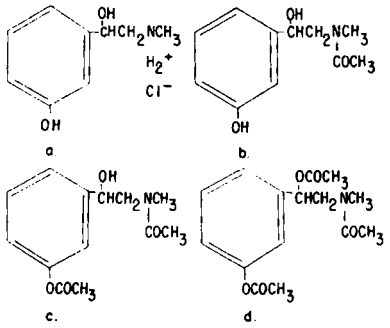

Fig. 1.—Chemical structures of phenylephrine and its synthetic acetyl derivatives: *a*, phenylephrine hydrochloride; *b*, *N*-monoacetylphenylephrine; *c*, *O,N*-diacetylphenylephrine; *d*, triacetylphenylephrine.

breakdown pathway is frequently impossible to predict. With heated solutions of phenylephrine hydrochloride and tetracaine, Schriftman (1) reported the presence of unknown breakdown products which were not found in solid formulations. Paper chromatographic procedures were utilized for the separation of degradation products. Evaluating the available analytical methodology, Schriftman (1) pointed out that phenylephrine assay procedures were based on reactions involving the hydroxyphenol nucleus (2, 3) and failed to detect changes on the side chain of the molecule. To overcome this latter problem, Kelly and Auerback (4) developed an ion exchange procedure which reportedly separated phenylephrine from interferring ingredients and resulted in a higher degree of analytical specificity for phenylephrine stability studies.

In working with stability samples of multiingredient tablet preparations containing phenylephrine hydrochloride, this laboratory found that the secondary amine characteristic of phenylephrine was being destroyed. To facilitate studies concerning the cause of the degradation, analytical procedures were developed which were specific for secondary amine character in the mixtures studied, did not involve complex separations, and were quite rapid. It was found that acetylsalicylic acid (aspirin) was involved in the phenylephrine degradation. Studies were conducted to determine what factors con-

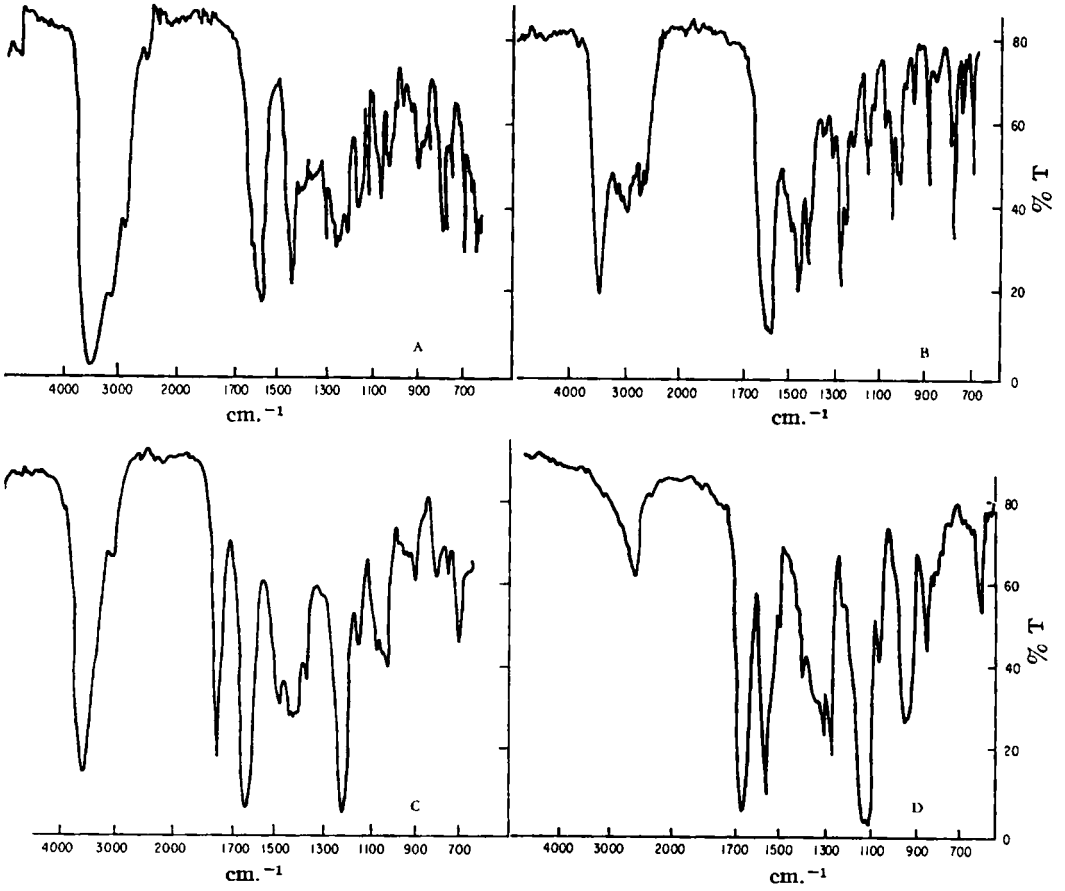

Fig. 2.—Infrared spectra of phenylephrine and its synthetic acetyl derivatives: *a*, phenylephrine hydrochloride; *b*, *N*-monoacetylphenylephrine; *c*, *O,N*-diacetylphenylephrine; *d*, triacetylphenylephrine. Spectra *a*, *b*, and *c* run in KBr disks. Spectrum *d* run in carbon tetrachloride with solvent compensation. All were recorded on a Beckman IR-4. Purity of synthetic derivatives was about 90%.

tributed to the degradation, the mechanism thereof, and the characterization of the breakdown products.

EXPERIMENTAL

Carbon Disulfide Assay for Secondary Amine Function.—All solvents and reagents were of analytical reagent quality and used without additional purification. Carbon disulfide:isopropyl alcohol:benzene mixture was prepared by pipeting 5 ml. of carbon disulfide and 25 ml. of isopropyl alcohol into a 100-ml. volumetric flask and diluting to volume with benzene. Ammoniacal copper solution was prepared according to Stanley, *et al.* (5). Acetic acid solution was a 30% v/v aqueous solution. As a standard stock solution, about 100 mg. of phenylephrine hydrochloride (Winthrop Laboratories) was accurately weighed, transferred to a 200-ml. volumetric flask, and diluted to volume with water. This solution was diluted exactly 1:10 with water as a working standard.

Procedure.—A representative tablet or powder sample equivalent to about 5 mg. phenylephrine hydrochloride was accurately weighed and transferred to a 100-ml. volumetric flask. The flask, to which about 50 ml. of water was added, was shaken 15 minutes. The mixture was diluted to volume with water, then filtered through Whatman No. 42 filter paper. Exactly 4 ml. of sample or working standard was transferred, in duplicate, to 50 ml. glass-stoppered centrifuge tubes. Exactly 10 ml. of carbon disulfide:isopropyl alcohol:benzene reagent and 1 ml. ammoniacal copper reagent was added to each tube. The tubes were shaken exactly 15 seconds on a Fisher clinical shaker; exactly 2 ml. of 30% acetic acid was added to each tube. The tubes were again shaken 15 seconds. The upper layer was filtered through Whatman No. 42 filter paper and the absorbances of the standards and samples read against benzene on a Beckman DU spectrophotometer at 434 mμ.

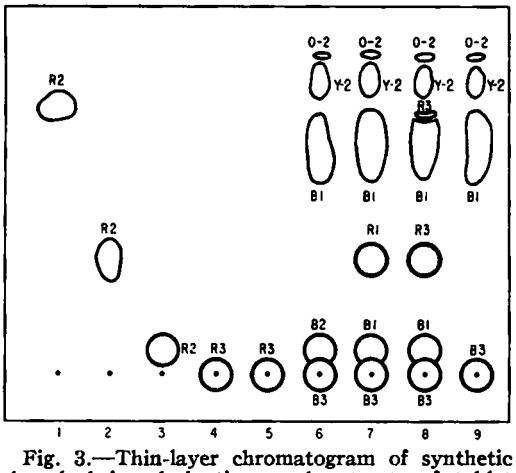

Fig. 3.—Thin-layer chromatogram of synthetic phenylephrine derivatives and extracts of tablet stability samples. Key: *1*, triacetylphenylephrine; *2*, *N*-diacetylphenylephrine; *3*, *N*-monoacetylphenylephrine; *4*, phenylephrine hydrochloride; *5*, phenylephrine base; *6*, tablet stored 6 months at room temperature—carbon disulfide assay method indicated 98% phenylephrine remaining; *7*, tablet stored 34 months at room temperature—carbon disulfide method indicated 14% loss, 4-aminoantipyrine method indicated 100% phenylephrine remaining although chromatogram indicated a trace of diacetylphenylephrine; *8*, tablet stored 2 months at 50°C.—carbon disulfide method indicated 93% loss of phenylephrine; *9*, a placebo containing all tablet ingredients except phenylephrine. Colors: R red, B brown, O orange, Y yellow, 1 faint, 2 moderate, 3 intense.

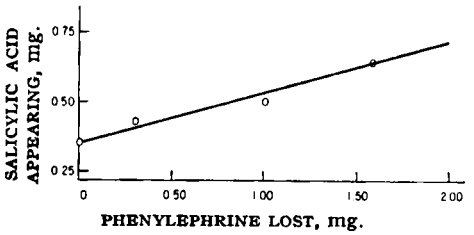

Fig. 4.—Decrease in phenylephrine activity plotted against increase in free salicylic acid for a multi-ingredient tablet containing phenylephrine hydrochloride and acetylsalicylic acid with storage at 70°C. for 4 weeks.

Synthesis of Acetylated Phenylephrine Derivatives.—*N*-Monoacetylphenylephrine (*N*-(*m*,β-dihydroxyphenethyl)-*N*-methylacetamine), Fig. 1*b*, was prepared according to Bretschneider (6).

N-Diacetylphenylephrine (*N*-(*m*-acetoxy-β-hydroxyphenethyl)-*N*-methylacetamide), Fig. 1*c*, was prepared according to Pratt (7).

Triacetylphenylephrine (*N*-(*m*,β-diacetoxyphenethyl)-*N*-methylacetamide), Fig. 1*d*, was prepared by dissolving about 10 Gm. of phenylephrine base in 30 ml. glacial acetic acid and 60 ml. acetic anhydride. The mixture was refluxed 6 hours, then placed in a glass-stoppered flask in a 105° oven for 7 days. The volume was reduced by evaporation at 50° under vacuum. The oily residue was dissolved in water and then extracted with several portions of carbon tetrachloride. The solvent was removed by evaporation on a steam bath. A thin-layer chromatogram of the product showed only one spot.

The identity of the mono and diacetyl derivates were confirmed by melting point. Further characterization of structure was obtained by infrared spectra (Fig. 2) and with thin-layer chromatograms which showed only single ingredient spots (Fig. 3).

Thin-Layer Chromatography.—A Brinkman model "S" applicator was used for all chromatograms. As an adsorbant, a 250-μ thickness of silica gel G (Brinkman) was applied. The best developing solvent was found to be the chloroform layer from an equilibrated mixture of chloroform, glacial acetic acid, and water (5:1:1). Color development was obtained by use of aqueous solutions of 0.3% *p*-nitroaniline dissolved in 1 *N* hydrochloric acid, 5% w/v sodium nitrite, and 20% w/v sodium carbonate.

Procedure.—For a synthetic acetylated compound about 10 mg. was dissolved in a mixture of 2 ml. ethanol and 0.5 ml. acetone. With tablets, two well ground tablets were thoroughly mixed with 2 ml. of ethanol and 0.5 ml. of acetone and the clear supernatant solution used. About 0.02 ml. of each solution was spotted to previously prepared and activated silica gel plates. After development the plates were air dried at room temperature. Detection of spots was accomplished by (*a*) spraying the plates with diazotized *p*-nitroaniline (to 25 ml. of

the acidic 0.3% *p*-nitroaniline, 1.5 ml. of sodium nitrite was added immediately prior to use), (*b*) drying the plates at 105° for about 10 minutes, and (*c*) spraying the plates with the 20% sodium carbonate solution.

RESULTS AND DISCUSSION

Analytical and Stability Factors.—Tablet preparations containing as active ingredients phenylephrine hydrochloride, acetylsalicylic acid, chlorpheniramine maleate, acetophenetidin, and caffeine were prepared and assayed for ingredient stability under accelerated temperature conditions. Only changes in acetylsalicylic acid and phenylephrine could be detected. For any one formulation held at an elevated temperature, the increase in salicylic acid content plotted against the decrease in phenylephrine content gave a linear relationship. Figure 4 shows a typical plot. It appeared, therefore, that there could exist some interrelationship between these degradations. Breakdown of phenylephrine was detected in the mixtures with the ion exchange procedure of Kelly and Auerbach (4) which further suggested that the secondary amine function of phenylephrine was involved.

Because of the involved nature of the Kelly and Auerbach procedure, efforts were directed toward other possible analytical procedures, particularly those that did not involve separation procedures. Of all the active ingredients and tablet excipients only phenylephrine contained a secondary amine function; this function was altered as indicated by the Kelly and Auerbach ion-exchange procedure. Specific secondary amine procedures were adaptable to the complex pharmaceutical mixtures under study. A modification of a procedure by Umbreit (8) worked. A second very rapid procedure with low blank values was developed and utilized for the work reported in this paper. This later method was based on a procedure reported for the assay of dimethylamine in methylamine by Stanley, Baum, and Gove (5), and prior ingredient separations were not needed. Evaluation of changes on the hydroxyphenol nucleus of the phenylephrine molecule was possible with the use of the 4-aminoantipyrine assay procedure of Koshy and Mitchner (9) which could also be applied to the tablet mixtures without prior separations.

Possible tablet ingredient interactions were further explored by submitting a series of powder mixtures to accelerated temperatures. These mixtures contained the active ingredients previously mentioned with the addition of various tableting agents such as binders, diluents, lubricants, and disintegration agents.

Mixtures were: [I] phenylephrine hydrochloride, acetylsalicylic acid (1:20); [II] phenylephrine hydrochloride, acetylsalicylic acid, magnesium stearate (10:200:1); [III] phenylephrine hydrochloride, acetylsalicylic acid, talc (1:20:1); [IV] phenylephrine hydrochloride, starch, magnesium stearate (50:150:1); and [V] phenylephrine hydrochloride, chlorpheniramine maleate, ascorbic acid, acetophenetidin, caffeine, starch (5:10:25:150:30:2). Analysis for secondary amine function with exposure to 70° for extended periods of time gave the results shown in Fig. 5.

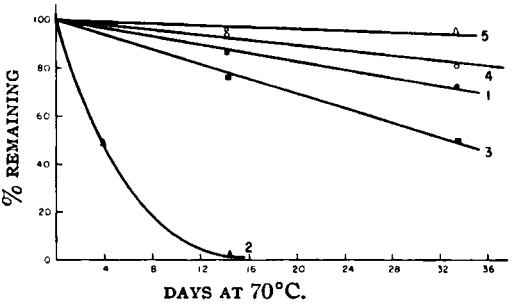

Fig. 5.—Decrease in secondary amine of phenylephrine in powder mixtures with storage at 70°C. Assay by carbon disulfide method. Key: *1*, phenylephrine hydrochloride, acetylsalicylic acid; *2*, phenylephrine hydrochloride, acetylsalicylic acid, magnesium stearate; *3*, phenylephrine hydrochloride, acetylsalicylic acid, talc; *4*, phenylephrine hydrochloride, starch, magnesium stearate; and *5*, phenylephrine hydrochloride, chlorpheniramine maleate, ascorbic acid, acetophenetidin, caffeine, starch.

TABLE I.—PHENYLEPHRINE CONTENT (MG./TABLET) AFTER STORAGE AT 70°C.

	Carbon Disulfide Method	4-Aminoantipyrine Method
Original, days	4.41	4.48
6	4.12	4.30
9	3.46	4.10
12	2.87	3.72
17	2.67	3.61
21	1.81	...
30	1.44	3.01

Combinations of phenylephrine hydrochloride and acetylsalicylic acid showed the greatest rates of phenylephrine degradation. With the addition of magnesium stearate, the breakdown was markedly accelerated. Very little phenylephrine was degraded in Mixture I with only phenylephrine hydrochloride and acetylsalicylic present and very little free salicylic acid was formed. Large amounts of free salicylic acid were formed in stability samples of Mixture II, which differed only from Mixture I by the addition of magnesium stearate. Previously, the effect of magnesium stearate in accelerating the breakdown of acetylsalicylic to salicylic acid and acetic acid in aspirin tablet formulations was shown by Ribeiro, *et al.* (10). It was concluded that breakdown of acetylsalicylic acid was prerequisite to the degradation of phenylephrine. The probable route of phenylephrine degradation was postulated as an acetylation of the secondary amine function with a possible contributory action from magnesium stearate since Castello and Mattocks (11) have shown that magnesium stearate can react with amine salts to release the free base. The free base of phenylephrine would be expected to be readily acetylated.

A multi-ingredient tablet formulation containing phenylephrine hydrochloride, acetylsalicylic acid, and magnesium stearate was stored at 70° and analyzed periodically for its phenylephrine content by both the carbon disulfide assay described in this paper and the 4-aminoantipyrine method (9). The results obtained are shown in Table I.

The reaction of 4-aminoantipyrine with a phenol

such as phenylephrine depends upon the *para* position to the phenyl group being unsubstituted (12). Loss of phenylephrine activity by attack only on the secondary amine portion of the side chain would not, therefore, be picked up by the 4-aminoantipyrine assay. A comparison of the results of the two assay techniques as shown in Table I suggested that both the phenolic portion of the phenylephrine and its secondary amine were being attacked under accelerated conditions but at considerably different rates. The secondary amine was more susceptible to attack. This was confirmed with samples held under less severe conditions. Similar tablet formulations held 2 months at 50° showed a 36% loss of phenylephrine by the 4-aminoantipyrine and 93% loss by the carbon disulfide assay. On samples stored at room temperature, loss of phenolic activity could not be detected after 34 months and secondary amine loss was 14% at that time.

Identification of Phenylephrine Degradation Products.—The identities of the degradation products were established by thin-layer chromatography of stability samples of tablets. The R_f values of spots not present in fresh samples were identical with the synthetic samples of mono, di, and triacetylated phenylephrines. Structures of derivatives are shown in Fig. 1. A thin-layer chromatogram of the three synthetic phenylephrine derivates compared to extracts of stability samples of multi-ingredient tablets containing phenylephrine hydrochloride and acetylsalicylic acid is shown in Fig. 3. From the chromatographic results it was apparent that (*a*) *N*-monoacetylphenylephrine formed under relatively mild conditions and in a short time, (*b*) *O*, *N* diacetylphenylephrine formed in addition to the monoacetyl compound under more severe conditions or extended periods of time at room temperature, and (*c*) all three acetylated compounds formed under severe accelerated stability conditions.

A number of different solvent systems for the thin-layer chromatography of phenylephrine and its degradation products were evaluated with the best system as previously described. Although not all of the systems yielded resolution of all of the degradation products, it was possible to confirm comparatively the identity of the unknown spots that did appear with the synthetic phenylephrine derivatives.

Oxidative Degradation of Phenylephrine.—Both the 4-aminoantipyrine method and the carbon disulfide assay methods were checked regarding applicability for the detection of oxidative degradation. Aqueous solutions of phenylephrine hydrochloride oxidized by boiling or by the addition of either hydrogen peroxide or manganese dioxide showed loss of phenylephrine of the same magnitude by either procedure.

SUMMARY

Formulations containing both phenylephrine hydrochloride and acetylsalicylic acid were found susceptible to loss of phenylephrine activity.

Degradation of phenylephrine resulted from acetylation, with acetylsalicylic acid providing the acetyl function. Breakdown products were identified as the mono, di, and triacetylated phenylephrine derivatives.

Tableting agents such as magnesium stearate which could accelerate the breakdown of acetylsalicylic acid were found to be contributory agents to a rapid loss of phenylephrine.

An assay was developed to detect loss of secondary amine activity in phenylephrine. With complex mixtures, specific functional group analysis could detect degradation on either the intact hydroxyphenol portion of the phenylephrine molecule or on the secondary amine of the side chain.

Oxidative degradation of phenylephrine could be detected by either the 4-aminoantipyrine or carbon disulfide method.

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