pretreated with reserpine or α methyldopa. Depletion of catecholamines with reserpine or α methyldopa (12) facilitated blockade of the effects of cryptenamine in atropinized animals, whereas guanethidine which does not induce depletion of catecholamines from the adrenal medulla (13) could not block the cryptenamine effects in animals pretreated with atropine sulfate. Therefore, it appears that nonparasympathetic efferent pathways involved in the reflex hypotension produced by cryptenamine may exert their action through the release of catecholamines from the adrenal medulla.

Since the effects of epinephrine are predominantly on α receptors (14), a sensitization of β receptors could mask any pressor effect that might result from epinephrine. The data obtained indicate a sensitization of β adrenergic receptors following the administration of cryptenamine.

It is apparent that there are many mechanisms in the complex phenomena of cryptenamine-induced hypotension. The results of this investigation which included the ability of P-286, reserpine, α methyldopa, and adrenalectomy to inhibit the effects of cryptenamine suggest a role of catecholamines and the adrenal medulla. The reduction of the cryptenamine effects by pronethalol, potentiation of the isoproterenol effects in the cat nictitating membrane and isolated perfused hind limb of the dog, and potentiation of the epinephrine-induced depressor responses in the dog indicate a role of the β adrenergic receptors and suggest that cryptenamine sensitizes the β adrenergic receptors to circulating epinephrine.

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Formation of Acetylcodeine from Aspirin and Codeine

By A. L. JACOBS, A. E. DILATUSH, S. WEINSTEIN, and J. J. WINDHEUSER

A reaction is discussed which leads to the formation of acetylcodeine from aspirin and codeine. It is noted that the generally published methods of analysis will not differentiate between the two alkaloids. A method of separating and assaying the individual compounds is described. Furthermore, the dependence of the interaction on water is discussed.

Although an abundance of products are marketed which contain combinations of aspirin, phenacetin, caffeine, and codeine, little has been published as to the stability and reactivity of these systems. Studies (1, 2) have been conducted on the stability of aspirin *per se*, but little attention has been given to its effect on other compounds.

During the development of a capsule product containing aspirin, phenacetin, caffeine, itobarbital, and codeine phosphate,[1] thin-layer chromatography indicated the presence of an unknown product in some samples after aging. The present communication deals with an investigation of this reaction and indicates that under certain conditions acetylcodeine forms. The acetylcodeine which results from this interaction of aspirin and codeine cannot be detected by the normal analytical methods employed for the determination of codeine. A partition column separation technique of codeine from acetylcodeine is described.

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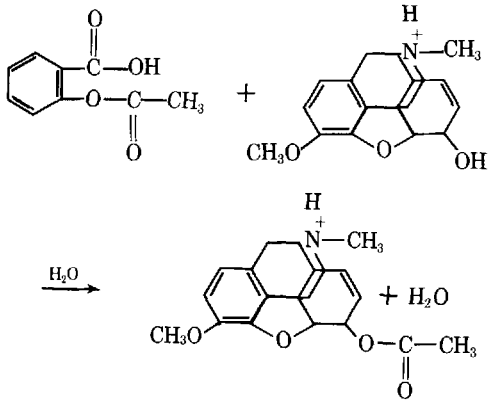
[1] Fiorinal plus Codeine Capsules, Sandoz Pharmaceuticals, Hanover, N. J.

RESULTS AND DISCUSSION

Isolation and Identification of the Reaction Product.—Based upon the possible reactants, it was speculated that an interaction might occur between aspirin and codeine (Scheme I).

Although the mechanism of the reaction has not been investigated, it might proceed by a classical transesterification or might be facilitated by an



Scheme I

anhydride as reported by Higuchi *et al.* (3) for a number of systems.

Mixtures of aspirin and codeine phosphate were placed into ampuls and water was added. The samples were sealed and heated at 60° for periods up to 4 weeks. At the end of regular time intervals the samples were fractionated using a thin-layer preparative chromatographic method. Aside from the aspirin, codeine, and salicylic acid, a new product was isolated. The isolation was carried out by scraping the silica gel from the plate in the area where the spot was evident and subsequent extraction with chloroform. The chloroform was evaporated to dryness leaving a white crystalline residue. The material was analyzed and compared with known acetylcodeine with the following findings.

Acetylcodeine, m.p. 131.5–132.5°; unknown m.p. 129.0–130°.

Anal.[2]—Calcd. for C, 70.4; H, 6.8; N, 4.1; O, 18.8. Found: C, 69.1; H, 6.7; N, 4.3; O, 18.6.

A mixed melting point (1:1) was 129.0°–131.0°. The ultraviolet and infrared spectra of the known and unknown were found to be identical. The R_f values of acetylcodeine, the unknown compound found in the aspirin–codeine mixture, and the unknown compound in the analgesic combination were identical in the systems described under *Experimental*. From these data one may conclude that this degradation product is acetylcodeine. A somewhat similar reaction was reported by Troup and Mitchner (4) involving the acetylation of phenylephrine.

Analysis of Mixtures Containing Codeine and Acetylcodeine.—As previously mentioned, it was noted during initial work on the analgesic combination that although a spot, now known to be acetylcodeine, appeared on the thin-layer chromatograms, no apparent loss of codeine was detected. The analytical technique used was a modification of the method proposed by Heuermann and Levine (5) for the separation of APC plus codeine mixtures. Ultraviolet measurements of the codeine fraction did not indicate any loss of the alkaloid, nor was any change apparent when the codeine fraction was titrated under nonaqueous conditions with perchloric acid. It is evident from Fig. 1, which shows the spectra of codeine and acetylcodeine, that the two are of the same order of magnitude. It is readily apparent that the *a* 1%, 1 cm. at 286 mμ of the compounds in chloroform are essentially identical (codeine = 55; acetylcodeine = 44), and, therefore, an ultraviolet method without prior separation would not be suitable for quantitative analysis.

The acetylation of codeine does not alter the basicity of the tertiary nitrogen and consequently does not affect the nonaqueous titration values.

Codeine and acetylcodeine can be separated by partition chromatography using a Celite support with the nonmobile phase being a 4% citrate buffer system at pH 3.5. At this pH the codeine is held on the column and the acetylcodeine is eluted with chloroform. The codeine can then be eluted with ammonia-saturated chloroform. Figure 2 is a typical chromatogram showing this separation of the products. Recovery of the acetylcodeine and codeine was shown to be quantitative.

From this it is evident that when assaying for codeine in an APC mixture according to the method of Heuermann and Levine (5), the codeine fraction should be passed through the citrate column to assay for acetylcodeine, after determining the presence of this degradation product by thin-layer chromatography.

Dependence of the Reaction on Water.—In order to ascertain the effect of moisture on the rate of the reaction, aspirin and codeine phosphate were mixed in the ratio of 100:15 w/w and placed into ampuls. Varying amounts of water were added and the ampuls sealed. A control sample was included to which no moisture was added. All ampuls were placed into a constant-temperature oven at 60° and heated for periods of time up to 22 days. At intervals, ampuls were withdrawn and assayed for aspirin, salicylic acid, codeine, and acetylcodeine.

The findings are shown graphically in Fig. 3. The data indicate that, as expected, the reaction is dependent on water in the lower moisture regions, but, however, at the higher levels the increase in the rate shows less of a dependency. This can most probably be attributed to the effect of the reverse

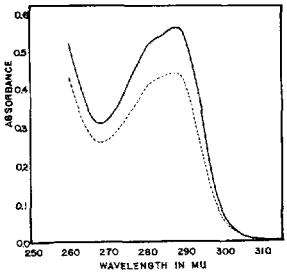


Fig. 1.—Ultraviolet spectra of codeine and acetylcodeine in chloroform. Key: ——, codeine; - - - - - -, acetylcodeine.

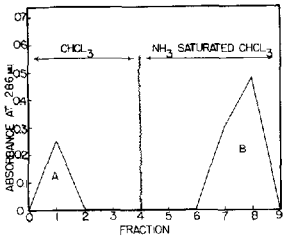


Fig. 2.—A typical chromatogram showing the separation of acetylcodeine (A) from codeine (B) by partition chromatography.

[2] The cooperation of Mr. Urs Stoeckli in performing the elemental analysis is gratefully acknowledged.

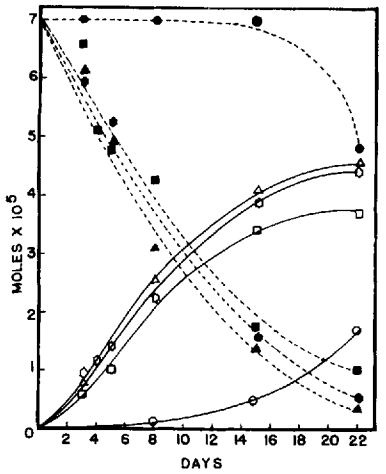


Fig. 3.—A composite representation of the degradation of codeine and formation of acetylcodeine under varying moisture conditions at 60°. Key: ●, < 0.1% moisture, codeine; ○, acetylcodeine; ■, 0.5% moisture codeine, □, acetylcodeine; ▲, 1.0% moisture, codeine; △, acetylcodeine; ⬢, 2.0% moisture, codeine; ⬡, acetylcodeine.

reaction which becomes more evident at the higher moisture concentrations.

It must be pointed out that the evaluation of such a heterogeneous system does not lend itself to a detailed kinetic analysis, but the results do indicate that moisture is essential for the reaction to proceed.

Analysis of commercial samples of the capsule product[1] stored at room temperature for 32 months indicated no evidence of acetylcodeine formation. These capsules were prepared in such a manner that the total moisture present was less than 0.2%. This bears out the findings with the simple aspirin–codeine mixture that at low moisture levels the reaction proceeds slowly even at elevated temperatures.

It should also be pointed out that based upon the work of Buckett *et al.* (6) the apparent activity and toxicity is not altered by the acetylation.

EXPERIMENTAL

Reagents

All chemicals used for the preparation of samples and analytical work were of U.S.P. or reagent grade, except for the acetylcodeine. The acetylcodeine was obtained as the hydrochloride (Merck), converted to the base, extracted with chloroform, and obtained in crystalline form by evaporation of the solvent, m.p. 131.5°–132.5°.

Analytical Procedures

Thin-Layer Chromatography.—*Plates.*—A 0.25-mm. layer of Silica Gel G (E. Merck, Darmstadt) was applied to the plates. The plates were air-dried for 10 min. and then heated in an oven for 45 min. at 120°. The plates were then stored in a desiccator until used.

Solvent System.—(*a*) Chloroform–acetone–diethylamine, 5:4:1 (7). (*b*) Chloroform–methanol, 10:1.

Spray Reagent.—One milliliter of 37% formaldehyde dissolved in 30 ml. of sulfuric acid (8).

Procedure and R_f Values.—Spot the equivalent of 100 mcg. of the alkaloid bases and develop over 10 cm. Dry the plate and spray with the reagent. Both codeine and acetylcodeine appear as purple spots. For nondestructive spraying, use distilled water to locate the compounds.

	Solvent	
R_f Values	(*a*)	(*b*)
Acetylcodeine	0.7	0.95
Codeine	0.4	0.60
Unknown[3]	0.7	0.95

Codeine Phosphate.—The nonaqueous titration as prescribed in the U.S.P. XVII was used to assay the codeine phosphate.

Aspirin.—(In mixture containing aspirin and codeine 100:15 w/w.) Place approximately 25 mg. of the mixture into a separator containing 30 ml. of chloroform and 10 ml. of 0.2 *N* sulfuric acid. Shake. Withdraw the organic layer and place into a 100-ml. volumetric flask. Repeat the extraction twice and bring the organic solution to volume with chloroform. Determine the absorbance of the solution at 280 mμ and compare with a known aspirin solution at the same wavelength.

Salicylic Acid.—Determined by the method of Levine (9).

Acetylcodeine and Codeine in Degraded Samples.—Adjust a 4% aqueous solution of citric acid monohydrate U.S.P. to pH 3.5 with 1 *N* sodium hydroxide. Mix 4.5 ml. of this solution with 7.5 Gm. of acid-washed Celite 545 (Johns-Manville Corp.) and pack into a 200 × 25 mm. chromatography tube previously plugged with glass wool. Top the Celite layer with a pledget of glass wool.

Add a 150-mg. sample of aspirin–codeine to a separator containing 10 ml. of 1 *N* sodium bicarbonate and 30 ml. of chloroform. After shaking, transfer the organic phase to the prepared column. Set a 100-ml. volumetric flask as a receiver beneath the column. Perform two additional chloroform extractions of the sodium bicarbonate layer adding each, in turn, to the column. Add sufficient water-saturated chloroform to the column to bring the eluate to 100 ml. This fraction contains the degradation product (acetylcodeine).

Elute the column with ammonia-saturated chloroform into a 100-ml. volumetric flask. This fraction contains the codeine. Read each fraction on a spectrophotometer at 286 mμ and compare with a standard.

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