distilling, later under vacuum. The residue was suspended in 100 ml. of distilled water and extracted with three 100-ml. portions of ether. The ether was then removed under diminished pressure and the alcohol recrystallized to analytical purity from an ethanol solution.

SUMMARY

The compounds evaluated indicate that the larger ring amine components in Mannich bases show pharmacological potentialities and should be included as amine components in series. More extensive work is in progress.

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Acetylation of Acetaminophen in Tablet Formulations Containing Aspirin

By K. T. KOSHY*, A. E. TROUP, R. N. DUVALL, R. C. CONWELL, and L. L. SHANKLE

The degradation of aspirin in commercial products containing acetaminophen was found to be accompanied by the formation of diacetyl-*p*-aminophenol (DAPAP) or *p*-acetoxyacetanilid. This reaction product was not detectable using conventional acetaminophen analytical procedures, but was detected qualitatively by means of thin-layer chromatography. A procedure utilizing partition–column chromatography and gas–liquid chromatography was developed for the determination of DAPAP in products containing aspirin, acetaminophen, and caffeine. Commercial products were found to contain various levels of DAPAP up to 4 mg./tablet. Magnesium stearate was found to accelerate the reaction markedly.

THE STABILITY of aspirin *per se* in pharmaceutical dosage forms has received considerable attention and the problem is widely recognized. However, reports of the interaction of aspirin with other drugs, such as amines and phenols, have only recently begun to appear in the literature. Troup and Mitchner (1) reported that phenylephrine underwent acetylation in tablets containing aspirin. Under accelerated conditions, the mono-, di-, and triacetylated products were formed. Even at 24° the secondary amine group

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was found to be acetylated to the extent of 14% after 34 months of storage.

Jacobs *et al.* (2), in a study of a capsule formulation containing aspirin and codeine, observed that acetylcodeine was formed but apparently only at elevated temperatures in the presence of moisture. No acetylcodeine was detected in commercial samples stored for 32 months at room temperature.

More recently, Schwartz and Amidon (3) recognized the possibility that aspirin might react with amino groups of proteins.

Preliminary work in these laboratories had indicated the possibility of an interaction between aspirin and acetaminophen (APAP) in pharmaceutical preparations. This paper summarizes the investigation of this interaction and shows, in particular, the extent to which it is taking place in commercial products.

Initial qualitative identification of the reaction product diacetyl-*p*-aminophenol (DAPAP) or *p*-acetoxyacetanilid was accomplished by means of thin-layer chromatography. Present assay methods for APAP, such as the column-partition method of Koshy (4), or a similar method by Levine and Hohmann (5), or the N.F. XII method (6) would not distinguish between APAP and DAPAP. Colorimetric methods which depend upon hydrolysis of APAP also lack specificity since DAPAP is readily hydrolyzed to *p*-aminophenol.

A quantitative method applicable to aspirin, APAP, and caffeine mixtures containing 0.01 to 20 mg. of DAPAP per tablet was developed. The procedure consists of the removal of caffeine, aspirin, and salicylic acid by a modification of the partition-column method developed by Levine (7) and removal of APAP and *p*-aminophenol (PAP) by a silicic acid column with water as the stationary phase. The residue obtained after passing the sample through the three columns was analyzed for DAPAP content by gas-liquid chromatography.

EXPERIMENTAL

Preparation of DAPAP—Twenty-seven grams of APAP was added to a mixture of 40 ml. acetic anhydride and 40 ml. reagent grade pyridine. This was refluxed 4 hr. on a steam bath. A precipitate formed when the mixture was allowed to cool to room temperature. The precipitate was collected by filtration and washed with 500 ml. of cold water to remove the pyridine and acetic anhydride. The residue was suspended in 150 ml. of hot water and heated almost to boiling. Small amounts of methanol were added until complete dissolution occurred. The solution was allowed to cool slowly to obtain the product, m.p. 152–156°. [Lit. m.p. 151–154° (8).]

Anal.—Calcd. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.85; H, 6.00; N, 7.54.

Qualitative Detection of DAPAP—One ground tablet was shaken thoroughly on a mechanical shaker with 10 ml. of chloroform and filtered, and the filtrate was evaporated to dryness under a stream of air. The residue was shaken with 1 ml. 3A alcohol and filtered. Twenty microliters of the filtrate was spotted on a 0.25-mm. Silica Gel G (Brinkmann Instruments, Inc.) plate previously activated by heating for 1 hr. at 105°. Twenty microliters of a reference solution of pure DAPAP (300 mcg./ml.) was also spotted for comparison. The plate was developed in a solvent mixture of chloroform–acetone–acetic acid (80:18:2) and allowed to dry. The DAPAP appeared as a yellow spot under long wavelength (350 mμ) U.V. light after about 10-min. irradiation with short wavelength (254 mμ) U.V. light.

For samples containing salicylamide the same chromatography system was used except aluminum oxide (neutral alumina AG7, Bio·Rad Laboratories) was used instead of silica gel.

Quantitative Determination of DAPAP—*Column Preparation*—All solvents used on the columns were water saturated. Three glass chromatographic columns, each 19 × 450 mm. (Fischer and Porter Co.), were used. The first column contained 10 Gm. of 100 mesh, analytical reagent grade silicic acid (Mallinckrodt Chemical Works) thoroughly mixed with 7 ml. of 4 *N* sulfuric acid. The column was washed with 100 ml. ethyl ether which had been freshly distilled over zinc and potassium hydroxide. The second column contained 10 Gm. silicic acid thoroughly mixed with 7 ml. water and was washed with 100 ml. chloroform N.F. (Allied Chemical, General Chemical Division). The third column contained 20 Gm. of diatomaceous earth[1] thoroughly mixed with 20 ml. 1 *N* sodium bicarbonate solution. The column was washed with 200 ml. chloroform N.F. It was necessary to acid wash the diatomaceous earth before it could be used. This was accomplished by adding a sufficient quantity of concentrated hydrochloric acid to the diatomaceous earth to form a thin slurry. The mixture was heated on a steam bath for about 1 hr. The diatomaceous earth was then washed free of acid with distilled water and dried for 16 hr. at 105°.

Sample Preparation—Five noneffervescent or two effervescent tablets were ground and shaken with 20 ml. of chloroform for about 5 min. The mixture was filtered through Whatman No. 541 filter paper into a 25-ml. volumetric flask and brought to volume by washing the residue with a small amount of chloroform.

Column Operation—Five milliliters of the sample solution together with 10 ml. of ether were added to the first column. When the liquid level reached the top of the silicic acid column, 10 ml. of ether was added. When the liquid level again reached the top of the silicic acid, 50 additional ml. of ether was added. The entire eluate was collected in a 150-ml. beaker. The eluate was evaporated to dryness using a current of air. (*Caution:* no heat.) The residue was dissolved in about 10 ml. of chloroform and transferred quantitatively to the second column using an additional 10 ml. of chloroform to rinse

[1] Marketed as Celite 545 by the Johns-Manville Corp., New York, N. Y.

the beaker. The rinsings were added to the column after the liquid just reached the top of the silicic acid. Another 50 ml. of chloroform was added to the column and the entire eluate was collected in a 150-ml. beaker and diluted to 100 ml. to reduce the aspirin concentration for more effective separation. The eluate was transferred quantitatively to the third column. When the solution level reached the top of the diatomaceous earth column, 100 ml. of chloroform was added in small portions. The eluate was collected in a 250-ml. beaker and evaporated down to a few milliliters on a steam bath. The concentrate was quantitatively transferred to a small vial and the evaporation was continued to dryness. The amount of DAPAP in the residue was determined by gas–liquid chromatography.

Gas–Liquid Chromatography—An F & M model 810 gas chromatograph with a flame ionization detector was used with an aluminum column $1^1/_2$-ft. by $^1/_4$-in. o.d. containing 5% Carbowax 20 M (Union Carbide Corp.) on Chromosorb G 70/80 mesh (Johns-Manville). The column temperature was 225° and both detector and injection port temperatures were 275°. Gas flow rates were: helium, 60 ml./min.; hydrogen, 65 ml./min.; and air, 300 ml./min. The sample residue was dissolved in 1–6 ml. of acetone N.F. depending upon the DAPAP content. Then, depending upon DAPAP concentration, 10 to 30 μl. of the sample was injected using a Beckman liquid sampling syringe. The amount of DAPAP was determined by comparison of sample peak height with a standard injected concurrently.

Salicylic Acid Determination—Salicylic acid was determined according to the method reported by Gold and Campbell (9).

RESULTS AND DISCUSSION

Initial qualitative evidence for the occurrence of a reaction between aspirin and APAP was obtained by thin-layer chromatography. A mixture of aspirin, APAP, and magnesium stearate with 1% water added was stored for 2 weeks at 50°. A portion of the sample was dissolved in ethyl acetate and passed through a diatomaceous earth column containing sodium bicarbonate as stationary phase to remove aspirin and salicylic acid. The effluent was evaporated to dryness using a current of air, the residue redissolved in acetone, and 20 μl. spotted on a silica gel plate. Development was with chloroform–ethyl alcohol–acetic acid (88:10:2). A spot was revealed by iodine vapor which did not correspond to salicylic acid, aspirin, or APAP. It was postulated that DAPAP was formed. Another solvent system, as described under *Experimental*, was developed which would separate DAPAP from other materials in commercial samples containing aspirin, APAP, and caffeine and two possible degradation products found in such mixtures, salicylic acid and *p*-aminophenol (PAP). Results are shown in Fig. 1. Samples 2 and 3 established that DAPAP could be separated from the other ingredients. Although the spot in sample 4 at R_f 0.52 is slightly lower than the DAPAP known, it was readily recognizable as DAPAP because of the yellow color exhibited by DAPAP under U.V. irradiation. Subsequent analysis of this same sample indicated 1.8 mg. of DAPAP per tablet.

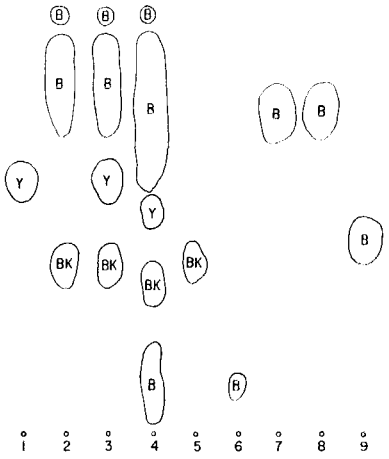

Fig. 1—Thin-layer chromatogram of aspirin, APAP, caffeine mixtures, and possible degradation products. Key: 1, DAPAP; 2, aspirin, APAP, caffeine mixture; 3, 2 plus DAPAP; 4, product A; 5, APAP; 6, PAP; 7, aspirin; 8, SA; 9, caffeine. Color under U.V. irradiation; B, blue; BK, black; Y, yellow.

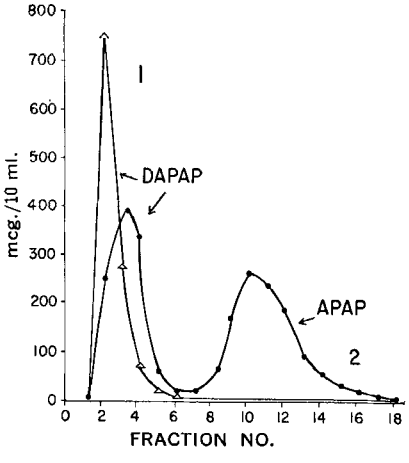

Fig. 2—Elution curve of APAP and DAPAP from silicic acid–water column. Key: 1, chloroform elution; 2, ether elution.

A procedure utilizing both partition column chromatography and gas chromatography was developed for the determination of DAPAP in products containing aspirin, APAP, and caffeine. The procedure involved removal of caffeine using a silicic acid–sulfuric acid column, APAP and PAP using a silicic acid–water column, and aspirin and salicylic acid by means of a diatomaceous earth–sodium bicarbonate column. DAPAP content in the final eluate was determined by gas chromatography. It was attempted, initially, to determine DAPAP in the eluate spectrophotometrically using its absorbance at 246 mμ. However, traces of aspirin coming through in the eluate caused erratic recoveries; whereas no interference was found using gas chromatography.

Figure 2 shows the elution of APAP and DAPAP from the silicic acid–water column. When the

Table I—Recovery of DAPAP from Tablet Mixtures[a] Containing Aspirin, APAP, and Caffeine

DAPAP Added, mcg.	Found Analyst A	Found Analyst B	Av. % Recovery
20830	20700	20430	98.8
6096	6020	5980	98.5
2083	1950	1950	93.6
1002	977	961	96.7
601	591	604	99.4
401	407	394	99.9
200	196	...	98.0
100	105	104	104.5
75.2	80.5	81.8	107.9
52.1	53.7	...	103.1
25.0	27.5	23.3	101.6

[a] Each mixture contained 324 mg. aspirin, 162 mg. APAP, and 33 mg. caffeine.

column is eluted with ether the DAPAP comes off first, followed closely by the APAP. However, if chloroform is used as the eluant, the DAPAP elution is sharper and the APAP is completely retained. This column could be used to determine DAPAP as a contaminant in APAP.

One discovery noted during the course of developing the assay is worthy of note. After a few inconsistent high recoveries, each step in the procedure was critically examined and it was found that DAPAP was formed during the evaporation step of the eluate from the first column since at that time a steam bath was being used. This phenomenon emphasizes that heat should be avoided prior to aspirin–APAP separation.

Recovery studies were performed on a typical formulation containing aspirin, APAP, caffeine, and various common lubricants and excipients. Known amounts of DAPAP in chloroform were added to the powder mixture prior to analysis. Results are shown in Table I. It is apparent that this analytical method is applicable to a wide concentration range of DAPAP limited only by its solubility in chloroform (about 4% w/v). The high recoveries for samples containing less than 100 mcg. may be attributed to the presence of trace impurities, since it was necessary to operate the gas chromatograph at high sensitivities for low DAPAP levels. The over-all accuracy and precision were probably as good as could be expected, especially in view of the complexity of these mixtures.

Commercial products, of unknown age, containing aspirin and APAP, were purchased from retail outlets and analyzed for DAPAP and salicylic acid (SA) content. Results are shown in Table II. As can be seen, aspirin–APAP interaction is taking place to varying extents in the samples analyzed. Product *A*, an effervescent tablet, was found to contain considerable quantities of DAPAP ranging from 1.24–4.13 mg./tablet. Product *B*, a press-coated tablet, contained lower levels of DAPAP ranging from 36–140 mcg. per tablet. Product *C*, a compressed tablet, contained only trace quantities of DAPAP. Product *D*, a compressed tablet, contained salicylamide which interfered in the analysis of salicylic acid and DAPAP. However, using the eluate from the partition–column chromatography procedure, DAPAP could be detected qualitatively using thin-layer chromatography in two samples of this product. By running various levels of a standard DAPAP solution simultaneously with the samples, the DAPAP content of the samples was estimated to be 15 mcg./tablet.

Inspection of the data in Table II indicates a relationship between the rate of formation of SA and the rate of formation of DAPAP. To study this further, tablets of product *A* from the same batch were heated for 6 weeks at 50° and analyzed weekly for SA and DAPAP content. Results, shown in Fig. 3, indicate a linear relationship between the two degradation products. The same trend exists in various samples stored at ambient conditions, although at lower SA and DAPAP levels the correlation is not so good.

The effect of various lubricants on aspirin stability has been the subject of numerous investigations. Alkaline earth stearates are reportedly responsible for poor aspirin stability in plain aspirin tablets (10) and aspirin, phenacetin, and caffeine combina-

Table II—DAPAP and SA Content of Commercial Products Containing Aspirin and APAP

Product	Comp.	Sample	SA, mg./Tab.	DAPAP, mg./Tab.
A	324 mg. aspirin 130 mg. APAP	1	32.4	1.24
		2	45.0	1.40
		3	31.4	1.64
		4	25.5	1.77
		5	49.3	1.90
		6	37.7	2.84
		7	50.2	3.57
		8	44.5	3.70
		9	62.7	4.13
B	227 mg. aspirin 194 mg. APAP 33 mg. caffeine	1	0.72	36
		2	1.33	52
		3	1.57	96
		4	1.48	103
		5	1.01	140
C	230 mg. aspirin 125 mg. APAP 30 mg. caffeine	1	0.69	0
		2	0.37	5
		3	0.31	10
D	Aspirin APAP Caffeine Salicylamide	1	...	Detected qualitatively
		2	...	Detected qualitatively

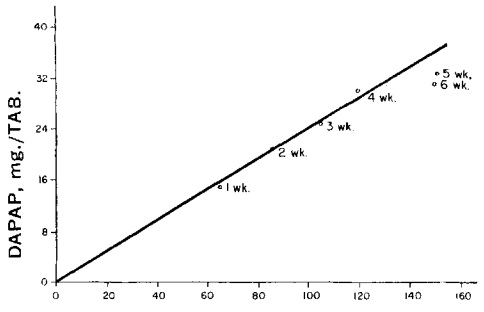

Fig. 3—Product A stored at 50°.

tions (11, 12). Magnesium stearate also has been shown to accelerate the formation of acetylphenylephrine in aspirin–phenylephrine combinations (1). Consequently, the relative effects of water, stearic acid, and magnesium stearate on the rate of formation of SA and DAPAP in aspirin–APAP mixtures were studied. Powder mixtures containing (*a*) 3.25 Gm. aspirin, 1.75 Gm. APAP, and 0.05 Gm. water; (*b*) mixture (*a*) plus 0.05 Gm. stearic acid; and (*c*) mixture (*a*) plus 0.05 Gm. magnesium stearate were sealed in small bottles and stored for 4 weeks at 50°. Subsequent DAPAP analysis of these mixtures indicated 67 mcg./Gm. in (*a*), 50 mcg./Gm. in (*b*), and 48.4 mg./Gm. in (*c*). Thus, magnesium stearate had a marked accelerating effect on the rate of DAPAP formation. Consequently, this lubricant is contraindicated in such formulations.

The pharmacological significance of the presence of DAPAP in these products is not known since information in the literature concerning this compound is scanty. Stockelbach (13) disclosed in a U. S. patent that DAPAP has valuable medicinal properties particularly as an antipyretic, antineuralgic, and sedative remedy. Charlier *et al.* (14) list DAPAP as one of the compounds studied for influence on bile secretion and toxicity, but did not give any data for DAPAP.

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Micellar Solubilization of Testosterone I

In Aqueous Solutions of Polysorbates

By ARVIND L. THAKKAR and NATHAN A. HALL

The solubility of testosterone in aqueous solutions of partially purified polysorbate 20, 40, and 60 was determined by measuring the area under the ultraviolet spectral peaks. There was solubilization below the critical micelle concentration. A linear relationship was observed between the amount of testosterone solubilized and the per cent polysorbate concentration. Polysorbate 60 exhibited the greatest solubilizing capacity and polysorbate 20 the least. The *Z*-value method for determining solvent polarity was employed to investigate the polarity of the environment in which testosterone was solubilized. At low polysorbate concentrations the *Z*-value was similar to that in water. As the polysorbate concentration increased, the *Z*-value decreased. This decrease in *Z*-value paralleled the increase in solubility of testosterone.

RECENT REVIEWS (1, 2) in pharmaceutical literature indicate that the phenomenon of solubilization continues to be of interest. In 1949 Ekwall and Sjöblom (3) published the first study on the solubilization of steroid hormones. They reported the solubility of testosterone in a 10% sodium oleate, 20% sodium myristyl sulfate, and 20% sodium cholate solutions. Nakagawa (4) has measured the solubility of testosterone in 20% aqueous solution of polyoxyethylene sorbitan monolaurate containing about 12 ethyleneoxide groups. Sjöblom and co-workers (5–7), in their

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