Simultaneous GLC Determination of Acetaminophen, Dichloralantipyrine, and Isometheptene Mucate

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Abstract □ A rapid GLC assay for acetaminophen, dichloralantipyrine, and isometheptene mucate in capsules was developed. These substances are chromatographed directly, using phenyl methyl silicone gum as the stationary phase. The standard calibration curves are linear up to 14 mg/ml, with a lower limit of sensitivity of 2 mg/ml. This method is simple and rapid and does not require derivative formation.

Keyphrases □ Acetaminophen—GLC analysis in capsules with dichloralantipyrine and isometheptene mucate □ Dichloralantipyrine—GLC analysis in capsules with acetaminophen and isometheptene mucate □ Isometheptene mucate—GLC analysis in capsules with acetaminophen and dichloralantipyrine □ GLC—analyses, acetaminophen, dichloralantipyrine, and isometheptene mucate in combination capsules □ Analgesics—acetaminophen and dichloralantipyrine, GLC analysis in capsules with isometheptene mucate □ Adrenergics—isometheptene mucate, GLC analysis in capsules with acetaminophen and dichloralantipyrine

Among the reasons for the pharmaceutical popularity of acetaminophen is that it is a nonsalicylate analgesic and antipyretic. Dichloralantipyrine is also used as a hypnotic analgesic, while isometheptene is a sympathomimetic agent effective as adjunctive therapy in the treatment of peptic ulcer. A mixture of the three agents is formulated in a pharmaceutical dosage form that is effective in the relief of vascular, tension, and migraine headaches.

Acetaminophen has been assayed by spectrophotometric (1–4) and chromatographic (5–9) methods. One spectrophotometric method has been reported for the assay of dichloralantipyrine (10), but there is none for the determination of isometheptene mucate. The lack of information regarding the simultaneous determination of acetaminophen, dichloralantipyrine, and isometheptene mucate in capsules, together with the tedious extraction requirements and derivative formation required in other assays, led to this investigation.

EXPERIMENTAL

Materials—Acetaminophen[1], dichloralantipyrine[2], isometheptene mucate[3], and pure ethanol[4] (all analytical grade) were used.

Apparatus—GLC analyses were performed on a gas chromatograph[5] equipped with a dual flame-ionization detector. The column was coiled stainless steel tubing (240 cm × 3 mm i.d.) packed with 1% phenyl methyl silicone gum (OV-17) on high-performance Chromosorb WHP[6] (80–100 mesh).

Helium was used as a carrier gas at a flow rate of 25 ml/min. The flow rates of hydrogen and air were 25 and 30 ml/min, respectively. The temperature of both the injection port and the flame-ionization detector was 190°. The columns were conditioned at 200° for 24 hr before use. During analysis, the temperature was programmed as follows: 3 min of 70°, increased to 185° at 5°/min, and finally held for 5 min at 185°.

Assay Procedures—Into each of three glass-stoppered centrifuge tubes, weigh accurately 20 mg of the capsule formulation containing 325 mg of acetaminophen, 100 mg of dichloralantipyrine, and 65 mg of isometheptene mucate. Add to each tube 10 ml of a 50 μg % ethanol solution of tuaminoheptane sulfate[7]. Mix the contents of each tube for 2 min using a vortex mixer and then centrifuge for 5 min at 5000 rpm.

Transfer the ethanol solution quantitatively to another glass-stoppered tube and evaporate it in a water bath at 50° under a nitrogen stream. Redissolve the residue in 0.20 ml of pure ethanol and transfer it to a 0.2-ml injection vial[8]. Evaporate the ethanol again under nitrogen and redissolve the residue in 40 μl of pure ethanol; then cap the injection vial.

Samples of 1 μl were injected into the gas chromatograph with a 1-μl syringe[9]. Standard calibration curves were established with ethanol solutions of known concentrations (2, 4, 6, 8, 10, 12, and 14 μg/ml) of the individual drugs to be assayed. Computation was done with both peak area and peak height measurements. Reproducibility was better with the peak area than the peak height technique.

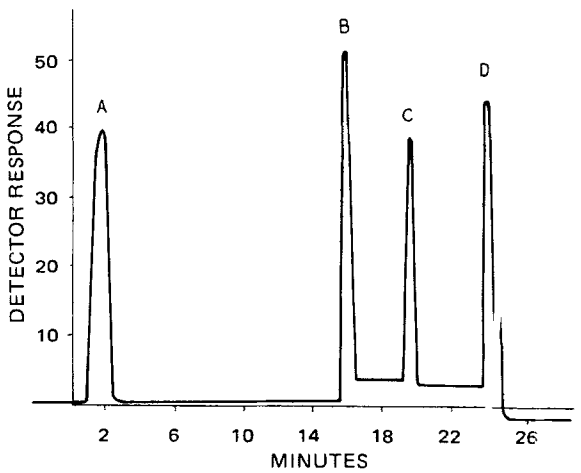

Figure 1—*Gas-liquid chromatogram. Key: A, dichloralantipyrine; B, acetaminophen; C, isometheptene; and D, tuaminoheptane sulfate (internal standard).*

RESULTS AND DISCUSSION

The standard calibration curves obtained by plotting the peak area ratio of acetaminophen, dichloralantipyrine, and isometheptene mucate *versus* their concentrations gave a straight line ($y = mx$) over the 2–14-μg/ml range with mean slope values of 0.66, 0.40, and 0.75, respectively.

Figure 1 shows a typical gas chromatogram of an acetaminophen, dichloralantipyrine, and isometheptene mucate mixture analyzed according to the described procedure. The retention times for dichloralantipyrine, acetaminophen, and isometheptene mucate were 3, 16, and 19 min, respectively; the retention time for the internal standard, tuaminoheptane sulfate, was 24 min. The overall recovery was constant from one test run to another. The results obtained with the procedure are illustrated by the examples in Table I.

The GLC determination of acetaminophen was accomplished either by measuring the unmodified drug directly (5, 6) or by converting acetaminophen to its trimethylsilyl ether or acetyl derivatives (7, 8, 11). McMartin and Street (11) reported that the reason for preparing a derivative of acetaminophen is that the unchanged drug is a highly polar compound that shows marked "tailing" during chromatography. However, there are two important disadvantages with acetaminophen de-

[1] McNeil Laboratories.
[2] Delmar Chemicals Co.
[3] Graham Laboratories.
[4] J. T. Baker Chemical Co.
[5] Hewlett-Packard model 7620A.
[6] Alltech Assoc. Inc., Arlington Heights, Ill.
[7] K and K Laboratories, Plainview, N.Y.
[8] Hewlett-Packard.
[9] Hamilton.

Table I—Arithmetic Mean of Determination of an Accurately Weighed Capsule Formulation

Compound	Labeled Amount, mg/ Capsule	Recovered Amount, mg/Capsule		Mean Percent
Acetaminophen	325	323.5		99.5
		322.8		99.3
		322.5		99.2
		Mean 322.9	Mean	99.3
Dichloralantipyrine	100	98.3		98.3
		97.6		97.6
		98.1		98.1
		Mean 98.0	Mean	98.0
Isometheptene mucate	65	63.20		97.2
		63.90		98.3
		Mean 63.5	Mean	97.7

rivatives. They are time consuming and are readily hydrolyzed by moisture even in the presence of excess reagents.

A comparison showed that the described method is more rapid than previously described GLC methods that required preparation of a derivative (7, 8, 11). It is not more rapid than other reported methods (5, 6), but the use of the 1% OV-17 on high-performance Chromosorb WHP eliminates the tailing and, therefore, increases the efficiency and accuracy.

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New Compounds: Synthesis of 2-Amino-5*H*-1,3,4-benzotriazepin-5-ones

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Abstract □ *N*-Methyl-2-aminobenzohydrazides, when treated with cyanogen bromide, were found to yield 2-amino-5*H*-1,3,4-benzotriazepin-5-ones.

Keyphrases □ 2-Amino-5*H*-1,3,4-benzotriazepin-5-ones—various derivatives synthesized □ Benzotriazepinones, substituted—various derivatives synthesized

Benzotriazepines as bioisosteric homologs of the well-established benzodiazepine psychotherapeutics have received considerable attention recently. Several synthetic methods and claims of sedative activity have been published (1–8) for the 1,3,4-benzotriazepine subclass, but a few earlier structural assignments (as benzotriazepines) were revised recently (9).

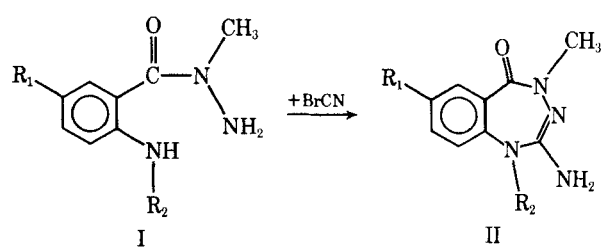
DISCUSSION

As a continuation of investigations on 1,4-benzodiazepin-3,5-diones derived from anthranilamides (10–12), this study reports the preparation of 2-amino-1,4-dihydro-5*H*-1,3,4-benzotriazepin-5-ones (II), a new type of 1,3,4-benzotriazepine obtained from the cyclization of anthranilohydrazides (I). By use of cyanogen bromide and a suitably methylated anthranilohydrazide, cyclization can be directed in an unambiguous fashion to the hydrobromide salts of the benzotriazepines, thus avoiding the classic pitfalls and uncertainties of structure that occur when alternative closure pathways are possible (9).

Anthranilohydrazides bearing a methyl on the amide-like nitrogen (*i.e.*, *N*-methyl-2-aminobenzohydrazides, I) were obtained by the opening of isatoic anhydride with methylhydrazine (13, 14). These hydrazides condensed instantaneously in chilled ethanol with cyanogen bromide (Scheme I) to give IIa–IIg in 36–85% yield. When the *ortho*-amino group was deactivated by conjugation to a nitro moiety, *i.e.*, If and Ig, condensation in refluxing dioxane was necessary to obtain a satisfactory yield.

The monomethylated benzotriazepines displayed four NH absorptions in the IR spectra between 3440 and 3080 cm^{-1}, while the dimethylated benzotriazepines (II, $R_2 = CH_3$) displayed only three absorptions between 3360 and 3080 cm^{-1}. Since tautomerism is possible in monomethylated compounds but not in IId, IIe, or IIg, these results may indicate a tautomeric equilibrium in the monomethyl isomers. No present structural evidence can eliminate their alternative formulation as 2-amino-3,4-dihydro-5*H*-1,3,4-benzotriazepin-5-ones.

In the PMR spectra, the N_4 methyl resonances appeared between 3.75 and 3.85 ppm; the N_1 methyl singlets were observed at 2.75–2.90 ppm. The NH resonances were variable in position, with the C_2 amino protons



Scheme I