A 3.8% UCC-W98 column was suitable for quantitative determination of higher molecular weight polyfunctional 9-acridine derivatives. For example, quinacrine hydrochloride had an R_t of 12.7 min. with a theoretical plate number (N) of 2853. This column was also suitable for the detection of the hydrolytic products of quinacrine (Compounds II and III). However, as indicated in Table I, these products have similar retention times, preventing the detection of one in the presence of the other at a column temperature of 200°. Note a decrease in the number of theoretical plates which, together with noticeable peak tailing, demonstrated a loss in column efficiency for these compounds. While excess tailing prevented the separation of Compounds I and II at lower column temperatures, this was possible for the lower molecular weight nonchlorinated parent compounds. Thus, while 9-aminoacridine could not be resolved from acridone at 200°, this could be accomplished at 182° (Table I).

Small amounts (100 ng.) of hydrolytic products could be detected in the presence of larger amounts (10 mcg.) of quinacrine hydrochloride on the 5.2% OV-17 column. On the 3.8% UCC-W98 column, the detection limit of quinacrine hydrochloride was about 100 ng. in the presence of excess (10 mcg.) hydrolytic products.

TLC fluorometric scanning was explored as an alternative method for the determination of quinacrine in the presence of its hydrolytic products. The development of suitable solvent systems is outlined in Table II. While chromatograms sprayed with 10% HCl followed by iodoplatinate reagent (4) visualized only the quinacrine as a weak blue-violet spot, compounds were readily detected by their natural fluorescence. The resolution of compounds was most satisfactory in Systems 3, 4, 5, 6, and 7, with mobility most satisfactory in Systems 1 and 7.

Of these two possibilities, System 7 was selected for quantitative TLC fluorometric scanning (Table III). Each compound was spotted at each concentration seven times, both as single compounds and as a mixture of compounds.

The mean of standard deviations for each compound determined separately and in mixture was 12.8%. The mean standard deviations for each chromatogram are listed in Table III. There was no significant difference in the standard deviations for a given compound for the determinations of compounds in mixture as compared to the determinations of single compounds. Factors responsible for the relatively high mean standard deviation include lack of uniformity of the thin-layer plates, the normal difficulty in reproducibility in plate spotting, and instrumental errors. In regard to plate uniformity, it should be noted that plates prepared in the laboratory were employed in this study in contrast to the more uniform commercially available plates.

In common with other *in situ* measurements of thin-layer plates (5, 6), it is not advisable to make comparisons between different plates nor to employ calibration curves. In general, linear relationships were obtained for calculated peak areas with respect to amounts applied. However, near the detection limits (5–10 ng.), there were deviations and the relationship did not always pass through the origin. Deviation from linearity owing to quenching was not observed for the maximum amounts (~100 ng.) used in this study.

Quantitative measurements are best made by multiple comparisons to known concentrations of standards on the same plate. It is important to emphasize that quantitative measurements could be made on a submicrogram level (10–100 ng.) with specificity provided simultaneously by TLC separation.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 17, 1971, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48104*

Accepted for publication June 1, 1972.

Supported in part by Research Grant AI 05817 from the National Institutes of Health, Bethesda, MD 20014

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Nature of Acetaminophen–Antipyrine Complex

J. C. DEARDEN

Abstract □ Acetaminophen and antipyrine form a single 1:1 complex which is shown to be stabilized through hydrogen bonding. IR spectroscopy shows both the —NH and —OH groups of acetaminophen and the carbonyl group of antipyrine to be involved in the complexation; the carbonyl group of acetaminophen is not. Methylation of either the —NH or the —OH group of acetaminophen is shown by phase equilibrium studies to prevent complexation with antipyrine.

Keyphrases □ Acetaminophen–antipyrine complex—nature of complexation □ Antipyrine–acetaminophen complex—nature of complexation □ Complexes—studies of complexation of acetaminophen–antipyrine

Antipyrine (phenazone) is known to complex with a wide variety of compounds and metallic ions (1); a number of workers (2–4) have reported complexation between antipyrine and phenols, with phenol itself forming a 1:1 complex with antipyrine (4). Complex formation has been reported not to occur between antipyrine and either acetanilide (5) or phenacetin (6), but a 1:1 complex of acetaminophen (*p*-acetamidophenol, *p*-hydroxyacetanilide) and antipyrine was recently patented (7) for its analgesic and antipyretic properties. This paper reports an investigation of the nature of that complex.

Table I—IR Spectra of Solid Acetaminophen, Antipyrine, and Complex

Stretching Band	In Acetaminophen	In Antipyrine	In Complex
O—H	3162 (broad)[a]	—	3070 (broad)[a]
N—H	3326	—	3258[a]
$N-CH_3$	—	3094	3099[b]
C=O	1659	1669	1684 1574

[a] Other minor peaks also present. [b] Band submerged in broad O—H band; identification tentative.

EXPERIMENTAL[1]

Materials—Most compounds used were obtained commercially and recrystallized. *p*-Hydroxy-*N*-methylacetanilide and its methyl ether were prepared according to the method of Julia and Bagot (8). The melting point of the former was 242°.

Anal.—Calc. for $C_9H_{11}NO_2$: C, 65.3; H, 6.67; N, 8.48. Found: C, 65.7; H, 6.76; N, 8.72.

The melting point of *p*-methoxy-*N*-methylacetanilide was 58°.

Anal.—Calc. for $C_{10}H_{13}NO_2$: C, 67.0; H, 7.26; N, 7.82. Found: C, 67.3; H, 7.47; N, 8.06.

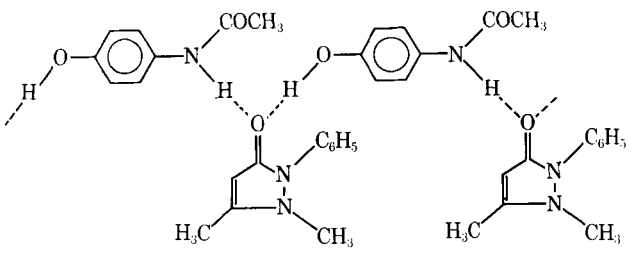
An NMR spectrum of this compound in deuterochloroform was consistent with the structure; methyl proton resonance peaks were observed at 5.94, 6.54, and 8.06τ. *p*-Hydroxy-*N*-methylacetanilide was too insoluble in $CDCl_3$ for a spectrum to be obtained, but a spectrum in deuteroacetone showed methyl proton resonance peaks at 6.79 and 8.21τ. No hydroxyl proton resonance could be detected, probably because of exchange with the enol form of the solvent.

Phase Diagrams—Eutectic points and solid–liquid curves were obtained from melting-range data.

RESULTS AND DISCUSSION

Melting-point determinations showed that acetaminophen and antipyrine form a single 1:1 complex with a congruent melting point of 108°. The complex is a colorless crystalline solid and can be formed by fusing equimolar mixtures of the two components or by crystallization from aqueous or other solutions containing approximately equimolar concentrations of each drug.

The complex was found to be too insoluble in hydrocarbon solvents for a UV spectrum to be obtained. The spectrum of the complex in ethanolic solution was found to be identical with the sum of the spectra of separate equimolar ethanolic solutions of acetaminophen and antipyrine. Since even relatively weak intermolecular forces such as hydrogen bonding and dipole forces have pronounced effects upon electronic absorption spectra (9, 10), it follows that the complex is completely dissociated in ethanol. Ekeblad (11) similarly found that an antipyrine–chloral hydrate complex, although soluble in chlorocarbon solvents, dissociates completely in aqueous solution. This is in marked contrast to the interaction between antipyrine and *p*-quinone, which gives rise to a stable charge-transfer complex showing marked spectral changes (12).



I

[1] UV spectra were determined on a Unicam SP-800 recording spectrophotometer, IR spectra were determined on a Grubb-Parsons Spectromaster and a Unicam SP.200 G spectrophotometer, and NMR spectra were determined on a Perkin-Elmer R.10 spectrometer. Solvents were commercially available spectroscopic grade materials and were used as received.

Table II—Phase Equilibrium Data for Mixtures of Antipyrine with Acetaminophen and Related Compounds

Mixture of Antipyrine and:	Eutectic Point(s) (Temperature; mole % Antipyrine)	Complex
Acetaminophen	104°; 40.5 83°; 71.5	1:1, m.p. 108°
p-Hydroxy-*N*-methylacetanilide	96°; 81	None
p-Methoxyacetanilide	68°; 48	None
p-Methoxy-*N*-methylacetanilide	46°; 8	None
Acetanilide	50°; 41	None
Phenacetin	73°; 53	None

The weakness of the acetaminophen–antipyrine complex suggests that it is formed through either dipolar or hydrogen-bonding interactions. Published IR studies indicated that hydrogen bonding is responsible for complexation between antipyrine and chloral hydrate (11), antipyrine and phenol (13), and aminopyrine and barbiturates (14). Acetaminophen is insoluble in tetrachloromethane and chloroform, but IR examination of a dilute (0.005 *M*) solution in dichloromethane showed that the free O—H stretching frequency at 3588 $cm.^{-1}$ and the free N—H stretching frequency at 3435 $cm.^{-1}$ were replaced, upon the addition of antipyrine to a concentration of 0.05 *M*, by a broad band centered at 3320 $cm.^{-1}$. Thus the interaction of acetaminophen and antipyrine in solution appears to occur through hydrogen bonding of both —OH and —NH groups, probably to the carbonyl group of antipyrine. However, the large excess of antipyrine over acetaminophen, necessary to induce association between the two compounds, meant that most of the antipyrine remained unassociated, and no shift of its carbonyl stretching frequency could be detected.

It does not necessarily follow from the above discussion that acetaminophen interacts *via* its —OH and —NH groups with antipyrine in the solid complex. An IR examination was, therefore, made of the solid in mineral oil, and the results are shown in Table I.

The O—H, N—H, and C=O stretching frequencies of pure solid acetaminophen are much lower than in dilute dichloromethane solution, indicating hydrogen bonding of both —OH and —NH groups to carbonyl. In the complex, the O—H and N—H stretching frequencies are lowered still further, indicating stronger interaction, which is consistent with the thermal stability and spontaneous formation of the complex. On the other hand, one of the carbonyl stretching frequencies is *higher* and the other much lower in the complex than in either of the components. The high frequency band is, in fact, very close to the free carbonyl stretching frequency of both acetaminophen (1690 $cm.^{-1}$) and antipyrine (1689 $cm.^{-1}$) in dilute (0.005 *M*) chlorocarbon solution. It is identified as that of acetaminophen for two reasons: (*a*) the band shape resembles that of acetaminophen much more than that of antipyrine and (*b*) since the carbonyl group of antipyrine must act as a proton acceptor in a hydrogen-bonded complex with acetaminophen, the C=O stretching frequency of antipyrine must fall upon complex formation. This can be demonstrated by the fact that the C=O stretching frequency of antipyrine of 1689 $cm.^{-1}$ at a concentration of 0.005 *M* in tetrachloromethane solution falls to 1658 $cm.^{-1}$ on the addition of phenol to 0.05 *M* to the solution.

It may reasonably be concluded that the carbonyl group of acetaminophen is *not* hydrogen bonded in the complex. That of antipyrine, on the other hand, is clearly very strongly hydrogen bonded, because its stretching frequency, relative to that of pure antipyrine, is extremely low ($\Delta\nu$ = 95 $cm.^{-1}$). It is suggested that this is because this group acts as a proton acceptor to both the —OH and —NH groups of acetaminophen (Structure I).

The involvement of the nitrogen atoms of antipyrine in complexation is regarded as extremely unlikely, since the steric effects of the adjacent hydrocarbon groups would tend to preclude this and also because the nitrogen atoms are less electronegative than the carbonyl oxygen. Furthermore, complexation has little apparent effect on the $N-CH_3$ stretching frequency. (The *N*-phenyl

stretching band could not be located and was presumably submerged in the large C—H stretching band.)

If the proposed structure for the complex is correct, it follows that methylation of either the —NH or —OH group (or both) of acetaminophen should prevent complexation with antipyrine. This was shown to be the case by melting-point determinations; neither congruently nor incongruently melting complexes were formed by the methylated derivatives of acetaminophen with antipyrine (Table II). The literature reports of lack of complexation between antipyrine and acetanilide or phenacetin (*p*-ethoxyacetanilide) were also confirmed.

It is perhaps surprising that since phenol forms a complex with antipyrine (4), *p*-hydroxy-*N*-methylacetanilide does not. The reason for this, which could be confirmed unequivocally only by X-ray crystallography, probably lies in the bulky nature of the acetamido group forcing a different crystal structure on the complex with antipyrine. Thus, *p*-hydroxy-*N*-methylacetanilide, because of its lack of a —NH group, is unable to complex in the way acetaminophen does; and by virtue of its steric properties, it cannot complex like phenol.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 20, 1972, from the *School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF, United Kingdom.*

Accepted for publication June 8, 1972.

The author thanks Mrs. L. Sheil and Mrs. D. A. Jones for technical assistance, and E. R. Squibb and Sons Ltd. for gifts of acetaminophen, antipyrine, and complex.

Oral Absorption of ^{14}C-Labeled Mepenzolate Bromide in Humans

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Abstract □ In a pilot study, a single oral dose of 25 or 30 mg. of mepenzolate bromide containing approximately 24 μc. of ^{14}C was given to each of four human volunteers and elimination of ^{14}C was followed in urine and feces for 4 or 5 days. From either a solid (capsule) or liquid dosage form, an average of 14% was eliminated in the urine (capsule: 7.0–20.2%, liquid: 3.1–21.1%), indicative of substantial oral absorption for an onium compound.

Keyphrases □ Mepenzolate bromide, radiolabeled—oral absorption, humans □ Absorption, oral—radiolabeled mepenzolate bromide, humans □ Radiolabeling—preparation of ^{14}C-mepenzolate bromide

It is well known that, as a generality, highly ionized organic compounds are not well absorbed following oral administration (1), and this appears true for some quaternary ammonium spasmolytic agents (2–4).

Mepenzolate bromide[1] (3-hydroxy-1,1-dimethylpiperidinium bromide benzilate), a potent postganglionic parasympathetic inhibitor (5), was reported (6) to be clinically useful in the treatment of motility disorders of the small and large bowels. Since no data have been reported on the oral absorption of this agent in humans, the present pilot study was undertaken to determine the absorption of a single oral dose as determined by excretion of ^{14}C in the urine. The assumption was made that little, if any, hydrolysis would occur in the intestinal tract. For this purpose, the compound was synthesized with a ^{14}C-label in one of the methyl groups. An additional objective was to compare the degree of absorption of the compound in solid dosage form (capsule) with that of a liquid solution formulation.

EXPERIMENTAL

Preparation of ^{14}C-Mepenzolate Bromide—*N*-Methyl-3-piperidyl benzylate, a commercial intermediate[2], was purified from water-insoluble material by solution in dilute hydrochloric acid, filtration with charcoal, and precipitation with dilute ammonium hydroxide. The solid was crystallized from hexane to form lustrous white crystals, m.p. 99–100°. Trial reactions of this tertiary amine with methyl bromide in acetone indicated almost quantitative formation of the insoluble onium salt in approximately 5 days at room temperature, m.p. 229–230° uncorr. [lit. (7) m.p. 234–236°]. Adequate pure tertiary amine was allowed to react in acetone with freshly prepared ^{14}C-methyl bromide[3]. The labeled product melted at 230–231° uncorr. and gave an IR spectrum identical to that of authentic cold material. Specific activity was 4.87 μc./mg. TLC [*n*-butyl al-

[1] Cantil, Lakeside Laboratories, Milwaukee, Wis.

[2] Supplied by Dr. Claude Judd, Lakeside Laboratories, Milwaukee, Wis.

[3] This reaction was carried out by Mallinckrodt-Nuclear, St. Louis, Mo., since ^{14}C-methyl bromide is prepared only on special order.