washing indicates little elution of the active ingredient as well as no hypsochromic effect. If salicylic acid is, however, substituted as the active ingredient as illustrated in Fig. 13, an interaction does result, but subsequent washing of the compressed sample causes the removal of part of the medicinal by the aqueous dispersion media. Substitution of salicylic acid as the active ingredient indicates that a greater degree of interaction is obtained by compression as compared to the previously discussed equilibrated salicylic acid system (Fig. 8). Here again, aqueous washing of the compressed sample (Fig. 13, B) results in partial removal of acid by the aqueous media. It is interesting to point out, however, that although the salicylic acid interaction is of a weaker variety, effects of varying compression pressures with respect to these complexes does result in proportional spectral changes; these are sufficiently greater than those observed as a result of particle size effects.

CONCLUSION

The data presented in this preliminary investigation indicate the presence of significant solid–solid interactions of medicinal agents studied with various adsorbents. Although the interactions are complex in nature, it is difficult at this time to assign a specific mechanism or mechanisms. However, generally speaking, these may be classified as a donor-acceptor type of interaction, although each system will have its individual spectral characteristics. Nevertheless, regardless of the mechanism(s) responsible for these complexes, the fact that they exist under equilibration, compression pressures, or other mixing conditions is of paramount importance. These interactions may certainly account for the discrepancies observed in blood levels and activities in various dosage forms. The reflectance technique, therefore, offers a means of confirming the existence of such interactions in solid dosage forms. Further studies are in progress in these laboratories concerning the interaction of various medicinal agents and adjuvants with respect to these solid–solid interactions.

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Reactions of a Secondary Amine in Chloroform

Implications for Drug Metabolism Studies

By J. L. LEELING, B. M. PHILLIPS, R. N. SCHUT, and O. E. FANCHER

Four new compounds were found to form in aged chloroform solutions of 1-(2-quinolyl)piperazine. Three of the compounds were identified, by comparison of thin-layer chromatographic behavior and infrared spectra with known compounds, as 1-formyl-4-(2-quinolyl)piperazine, 1-chlorocarbonyl-4-(2-quinolyl)piperazine, and 1,1′-oxomethylenebis[4-(2-quinolyl)piperazine]. Three new compounds were found to form in aged ethylene chloride solutions of 1-(2-quinolyl)piperazine, while only one new compound formed in aged methylene chloride solutions. It is concluded that the use of chlorinated hydrocarbons for extracting secondary amines from biological media should be approached with caution, especially if the extracts are allowed to stand for 24 hr. or longer.

THE COMPOUND, 1-(2-quinolyl)piperazine maleate (MA1291), is a new experimental oxytocic agent (1). During the course of thin-layer chromatographic experiments preliminary to studies of the biological disposition of the compound, it was found that chloroform solutions of the free base of MA1291 more than 1 day old contained from three to five components, depending on the age of the solution. In view of its potential significance, this phenomenon was investigated in an attempt to identify the new components and to gain some insight as to the reaction mechanisms. This report is presented to indicate the potential risk involved when chlorinated hydrocarbons are used to ex-

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TABLE I.—THIN-LAYER CHROMATOGRAPHIC BEHAVIOR OF DERIVATIVES AND AGED CHLOROFORM SOLUTIONS OF MA1291 FREE BASE

Solvent System[b]	Components of Aged Chloroform Soln.[c] A	B	C	D	E	MA1291 Free Base and Synthetic Derivatives I	II	IV	III
	Rf[a]								
1	0.00	0.15	0.60	0.84	1.00	0.00	0.62	0.82	1.00
2	0.00	0.18	0.39	0.57	0.84	0.00	0.42	0.57	0.85
3	0.46	...	0.77	0.85	1.00	0.46	0.81	0.83	1.00
4	0.64	...	0.92	0.92	0.97	0.65	0.91	0.93	0.97
5	0.00	0.17	0.63	0.90	1.00	0.00	0.64	0.94	1.00
6	0.78	...	0.88	0.95	1.00	0.78	0.90	0.95	1.00
7	0.56	0.12	0.66	0.88	0.95	0.55	0.65	0.89	0.93
8	0.26	0.76	0.87	0.91	0.91	0.26	0.85	0.93	0.92
9	0.57	0.31	0.52	0.75	0.81	0.56	0.52	0.74	0.81
10	0.11	0.37	0.66	0.74	0.81	0.12	0.66	0.74	0.81
11	0.08	0.42	0.65	0.73	1.00	0.10	0.61	0.71	1.00
12	0.71	0.44	0.88	0.88	0.96	0.71	0.88	0.90	0.98

[a] All runs were made at room temperature. The distance from the origin to the center of a spot was used in the calculation of the *Rf* value. [b] The following solvent systems were employed: 1, chloroform; 2, acetone–heptane (1:1); 3, *n*-butanol–ethanol–water (9:1:1); 4, methanol; 5, ethyl ether–chloroform–methanol (50:50:1); 6, chloroform–diethylamine (9:1); 7, benzene–ethyl acetate–diethylamine (7:2:1); 8, chloroform–ethyl acetate–methanol (2:2:1); 9, butylether–*n*-butanol–acetic acid (5:4:1); 10, butylether–*n*-butanol–28% ammonium hydroxide (15:5:1); 11, chloroform–acetone–diethylamine (5:4:1); 12, benzene–ethyl ether–acetic acid–methanol (120:60:18:1). [c] Freshly prepared solutions of MA1291 free base in chloroform were shown to contain only one component, which migrated in a manner identical to *A* and I above.

tract drugs from biological media in studies of the metabolism of secondary amines or tertiary amines which yield a secondary amine metabolite.

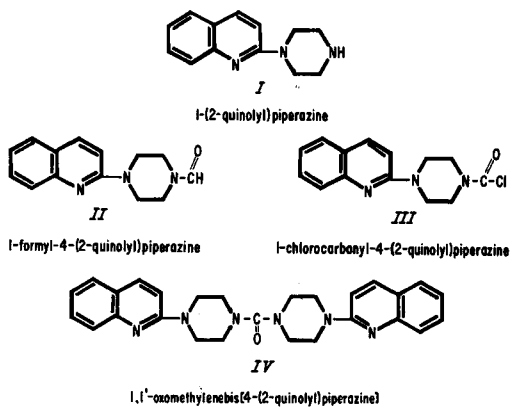
MATERIALS AND METHODS

Chromatography.—Qualitative ascending thin-layer chromatography was conducted with solutions of free bases of MA1291 and its derivatives on 5 × 20-cm. glass plates coated with a 250-μ layer of aluminum oxide GF_{254}.[1] Generally 10 μl. of 1 mg./ml. solutions were spotted. Twelve solvent systems were employed (Table I).

MA1291 and its derivatives were detected initially with ultraviolet light of either 254 or 366 mμ. Subsequently, the spots were made visible by spraying the plates with potassium iodoplatinate solution (2); reactive materials appeared immediately as light blue to maroon areas against a light brown background.

For preparative chromatography, 20 × 20-cm. glass plates coated with a 500-μ layer of aluminum oxide G[1] were used. Twenty 25-μl. aliquots of a 25 mg./ml. aged solution of MA1291 free base were spotted individually in a line 2 cm. from the bottom of the plate; plates were developed in acetone–heptane (1:1). After development, the bands corresponding to the various components were located under ultraviolet light, and marked and cut from the plates. After 20 plates were run in this manner, the accumulated fractions were packed into columns and the solutes eluted with methanol. The methanol eluates were evaporated to dryness *in vacuo* and the residues were subjected to infrared analysis. The yield of the three compounds isolated in this manner in no instance exceeded 20 mg.

Chemical Synthesis.—Compound II was prepared by heating 0.01 mole of compound I, m.p. 82–83°, with 50 ml. of formamide[2] over a steam bath for 2 hr. While standing in the cold for 48 hr., white needles crystallized which, upon recrystallization from methanol, melted at 177–179°. The infrared spectrum and nitrogen analysis (Table II) were compatible for 1-formyl-4-(2-quinolyl)piperazine.



Structure of MA1291 Free Base and Its Derivatives

Compound III was prepared by the dropwise addition of a solution of 0.01 mole of phosgene in 50 ml. of chloroform to a stirred mixture of 0.01 mole of MA1291, 100 ml. of chloroform, and 50 ml. of 20% NaOH. The reaction was run for 30 min. at 22°. The aqueous layer was then removed and discarded. The chloroform layer was dried by passing through a 1 × 15-cm. column of anhydrous sucrose, and the filtrate was evaporated to dryness under a stream of dry air. The product, after recrystallization from tetrahydrofuran–2-propanol, melted at 171–173°. The infrared spectrum, nitrogen analysis (Table II), and positive chloride test were consistent for 1-chlorocarbonyl-4-(2-quinolyl)piperazine.

TABLE II.—NITROGEN ANALYSIS OF SYNTHETIC DERIVATIVES

Compd.	% N Calcd.	Found
II	17.42	17.66
III	15.24	14.88
IV	18.56	18.52

[1] Supplied by E. Merck A. G., Darmstadt, Germany.
[2] Supplied by Matheson Coleman and Bell.

Compound IV was synthesized by adding dropwise a solution of 0.01 mole of MA1291 in 50 ml. of chloroform to a rapidly stirred slurry of compound III (0.01 mole) in 100 ml. of chloroform and 50 ml. of 20% NaOH. The reaction mixture was refluxed with constant stirring for 16 hr. and then allowed to cool to room temperature. The chloroform was removed, passed through a column of sucrose, and evaporated to dryness under a stream of dry air. The resulting pale yellow plates melted at 225–226° upon recrystallization from methanol–tetrahydrofuran. The infrared spectrum and nitrogen analysis (Table II) confirmed the structure of compound IV to be 1,1′-oxomethylenebis-[4-(2-quinolyl)piperazine].

EXPERIMENTAL

The chromatographic behavior of aged solutions of MA1291 free base in chloroform was compared to that of freshly prepared solutions and of the synthetically prepared derivatives. The results obtained (Table I) indicate that component *A* of the aged solution migrates identically to the free base of MA1291 (compound I), component *C* compares with compound II, component *D* compares with compound IV, and component *E* compares with compound III. When superimposed aliquots of an aged solution of MA1291 free base in chloroform, a freshly prepared mixture of MA1291 free base, and the synthesized derivatives were chromatographed, five spots were seen with R_f values identical to those seen when aged solutions alone were run. The identity of component *B* was not determined. This component was first observed on chromatograms after the chloroform solutions had aged for approximately 2 months and appears to increase in concentration as the solutions grow older.

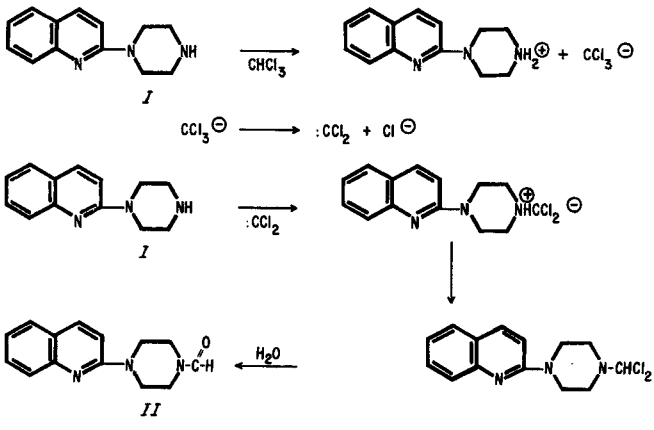
The materials isolated from preparative thin-layer plates were subjected to infrared spectral analysis and the spectra obtained were compared to those of the reference compounds (compounds II, III, and IV). The results of the comparison indicated component *C* to be compound II, component *D* to be compound IV, and component *E* to be compound III. In only one instance was it possible to compare melting points; component *E* did not significantly depress the melting point of pure compound III.

Solutions of MA1291 free base in methylene chloride and ethylene chloride were also examined for the formation of new compounds. After standing for more than 2 months, the methylene chloride contained MA1291 free base and a very low concentration of one other component. No attempt was made to identify this component. Aged ethylene chloride solutions, on the other hand, contained MA1291 free base plus three contaminants. Preliminary evidence indicates that two of the contaminants are probably 1-(2-chloroethyl)-4-(2-quinolyl)piperazine and 1,1′ - ethylenebis[4 - (2-quinolyl)piperazine]. However, when synthesis of the above compounds was attempted under Schotten-Baumann conditions from MA1291 and 1-bromo-2-chloroethane or ethylene bromide, respectively, the results obtained were unsatisfactory. In the former case, the yield obtained was satisfactory, but the major product underwent change when purification was attempted. When heated, the 1-(2-chloroethyl)-4-(2-quinolyl)piperazine probably forms a quaternary ammonium salt. In the latter synthesis the yield was so small that identification of the product was precluded. However, when the reaction mixtures were chromatographed in acetone–heptane (1:1), three spots, identical to those on chromatograms of aged MA1291 free base in ethylene chloride, were seen.

DISCUSSION

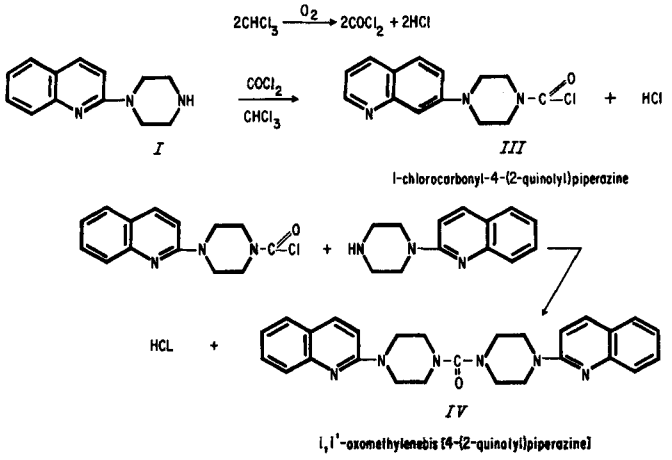
Three of the contaminants appearing in aged chloroform solutions of MA1291 free base have been identified as 1-formyl-4-(2-quinolyl)-piperazine, 1-chlorocarbonyl-4-(2-quinolyl)piperazine, and 1,1′-oxomethylenebis[4-(2-quinolyl)piperazine]. The nature of the contaminants suggests that their formation may proceed by two different mechanisms.

Compound II, 1-formyl-4-(2-quinolyl)piperazine, may be formed when chloroform reacts with MA1291 free base (Scheme I) to form dichlorocarbene which reacts with another molecule of MA1291 free base to form a dichloromethylene derivative. This hydrolyzes spontaneously in the presence of traces of water to yield the formyl



Proposed Mechanism for the Formation of 1-Formyl-4-(2-quinolyl)piperazine in Chloroform Solutions of MA1291 Free Base

Scheme I



Proposed Mechanism for the Formation of 1-Chlorocarbonyl-4-(2-quinolyl)piperazine and of 1,1′-Oxomethylenebis [4-(2-quinolyl)piperazine] in Chloroform Solutions of MA1291

Scheme II

derivative. This mechanism has been suggested for the formation of *N*-formylpiperidine in chloroform solution (3, 4).

Compound III, 1-chlorocarbonyl-4-(2-quinolyl) piperazine, may be formed by the reaction of MA1291 free base with phosgene present in the chloroform (Scheme II.) The 1-chlorocarbonyl-4-(2-quinolyl)piperazine probably then reacts with another molecule of MA1291 to yield 1,1′-oxomethylenebis[4-(2-quinolyl)piperazine] (IV).

Another contaminant appeared after the chloroform solution had aged for at least 4 weeks. It was not possible to identify this material, but it is known not to be 1-carboxyl-4-(2-quinolyl)piperazine, 1-carboxymethyl-4-(2-quinolyl)piperazine, 1-amino-4 - (2 - quinolyl)piperazine, or 1,1′ - methylenebis-[4-(2-quinolyl)piperazine]. Whether this compound is formed through either mechanism, or by another, is also unknown.

Aged solutions of MA1291 free base in ethylene chloride or methylene chloride also yielded contaminants—three in the former case and only one in the latter. None of these contaminants was positively identified. Heptane or ethyl ether solutions of MA1291 free base which stood for more than 3 months contained only the original compound as shown by thin-layer chromatography.

It is concluded that the use of chlorinated hydrocarbons of the type discussed to extract secondary amines from biological media must be undertaken cautiously. If extracts are to be left standing for any length of time, it should be determined in advance whether the compound in question will react with the solvent. Failure to do so may lead to mislabeling solvent–solute reaction products as metabolites. If the nature of the amine requires the use of chlorinated hydrocarbons for extraction, the authors' experience suggests that methylene chloride is probably the solvent of choice.

SUMMARY

Four new compounds were found to form in aged chloroform solutions of MA1291 free base. Three of the compounds were identified as 1-formyl-4-(2 - quinolyl)piperazine, 1 - chlorocarbonyl - 4-(2 - quinolyl)piperazine, and 1,1′ - oxomethylenebis[4 - (2 - quinolyl)piperazine]; the fourth product was not identified. Identification was accomplished by comparison of the infrared spectra and thin-layer chromatographic behavior of the new compounds with those of known compounds synthesized in this laboratory. The compounds formed in chloroform were of two types: (*a*) those which might be expected from the reaction of MA1291 free base with phosgene, an oxidation product of chloroform and (*b*) the *N*-formyl derivative which could be expected to form by the reaction of chloroform (*via* dichlorocarbene) and water with MA1291 free base.

In addition to MA1291 free base, aged ethylene chloride solutions of the material contained three new compounds, while only one new compound formed in aged methylene chloride solutions. None of these compounds was positively identified. New compounds failed to form in aged solutions of MA1291 free base in either heptane or ethyl ether.

It is concluded that the use of chlorinated hydrocarbons for extracting secondary amines from biological media should be approached with caution. This is especially true if the extracts are allowed to stand for 24 hr. or longer.

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