Synthesis of Diastereoisomeric 4-Dimethylamino-3-phenyl-2-butanols and Related Esters for Antimicrobial Evaluation

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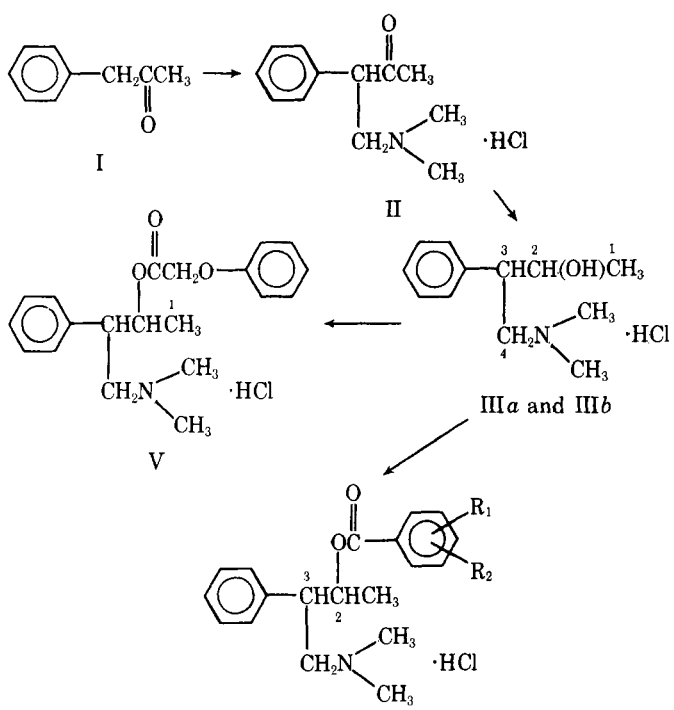
Abstract □ 4-Dimethylamino-3-phenyl-2-butanone was reduced to the corresponding diastereoisomeric alcohols, which were separated by fractional crystallization of the corresponding hydrochloride salts. The configuration of the diastereoisomeric alcohols was determined by PMR spectroscopy. The assignments were confirmed by a consideration of the mass spectral data obtained for the two alcohols. Acylation of the alcohols gave the corresponding esters. Antimicrobial evaluation of the compounds prepared showed that 4-dimethylamino-3-phenyl-2-butanone had a promising level of antifungal activity while the other derivatives showed either a low level of potency or were inactive.

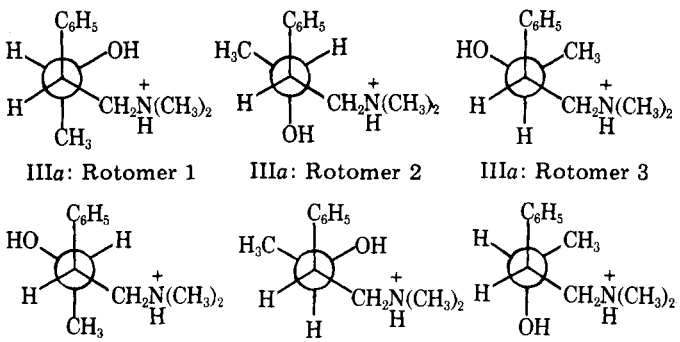
Keyphrases □ 4-Dimethylamino-3-phenyl-2-butanols—diastereoisomers and related esters, synthesized, antimicrobial activity evaluated □ Antimicrobial activity—evaluated in diastereoisomeric 4-dimethylamino-3-phenyl-2-butanols and related esters □ Structure–activity relationships—diastereoisomeric 4-dimethylamino-3-phenyl-2-butanols and related esters evaluated for antimicrobial activity

Various Mannich bases (β-aminoketones) have antimicrobial activity (1–4); reduction of the keto group in this series of compounds to the corresponding alcohol has been accompanied by an increase in pharmacological activity (5). The bioactivities of such alcohols may be improved by esterification, thereby masking the polar hydroxyl group and, consequently, facilitating transportation to a site of action.

With benzoate esters of alcohols derived from Mannich bases, the hydrolysis rates are influenced by the Hammett and Taft values of the nuclear substituents, and correlations between the hydrolysis rates and antimicrobial activity may emerge. Furthermore, acylation of these alcohols to the antifungal agent phenoxyacetic acid to produce esters capable of hydrolysis was of interest. As a continuation of a program on the synthesis and antimicrobial evaluation of various acyclic and cycloaliphatic Mannich bases and the related alcohols and esters (6–8), II–V (Scheme I) were prepared.



Scheme I



RESULTS AND DISCUSSION

The Mannich reaction between phenylacetone, paraformaldehyde, and dimethylamine hydrochloride occurs either at the methylene or methyl group of the ketone. The compound obtained gave the expected product, namely, the Mannich base II, as shown by PMR spectroscopy. Reduction of II with lithium aluminum hydride gave diastereoisomers III*a*, mp 142–143°, and III*b*, mp 93°. PMR spectroscopy of the crude reduction product showed that the ratio of III*a* to III*b* was approximately 60:40.

The elucidation of the relative stereochemistry of III*a* and III*b* was achieved by PMR spectroscopy. In diastereoisomers bearing a proton at both asymmetric centers, the vicinal coupling constants will be the average value for the various rotomer contributions. If, in the preferred conformation, the vicinal protons are *trans* to each other, the coupling constant of the adjacent protons is 8–12 Hz; with *gauche*-oriented protons, the average reported values are 1–4 Hz (9, 10).

An examination of the conformational free energy differences[1] of the groups at carbons 2 and 3 allows a prediction of the preferred rotomer. At carbon 2, the conformational free energy differences of methyl and hydroxyl groups are 1.7 and 0.7 kcal/mole, respectively (11, 12). At carbon 3, the conformational free energy difference of the phenyl ring is 3.1 kcal/mole (12). Although the literature appears to be deficient in assigning a value to the dimethylaminomethyl group, it is known that the

[1] The conformational free energy difference is defined as the negative of the free energy difference $\Delta G_x°$ corresponding to the conformational equilibrium constant K for a monosubstituted cyclohexane with the substituent, x, axial or equatorial.

Table I—Physical Properties of Benzoate Esters of 4-Dimethylamino-3-phenyl-2-butanol Hydrochloride

Compound	Yield, %	Melting Point	Molecular Formula	Analysis, % Calc.	Analysis, % Found	Mass Spectrum[a] P Calc.	Mass Spectrum[a] P Found	IR Spectra, cm^{-1} (C=O)
IV*a*	40	145–147°	$C_{19}H_{24}ClNO_2$	C 68.35 H 7.25	68.40 7.47	297	297	1715 (s)
IV*b*	69	204–205°	$C_{20}H_{26}ClNO_3$	C 66.01 H 7.20	65.80 7.40	327	327	1700 (s)
IV*c*	78	192–193°	$C_{19}H_{23}Cl_2NO_2$	C 61.96 H 6.29	61.70 6.59	331	331	1725 (s)
IV*d*	65	200–201°	$C_{19}H_{23}Cl_2NO_2$	C 61.96 H 6.29	61.60 6.62	331	331	1720 (s)
IV*e*	83	179–180°	$C_{19}H_{23}ClN_2O_4$	C 60.23 H 6.12	60.30 6.29	342	342	1725 (s)
IV*f*	81	207–209°	$C_{19}H_{22}ClN_3O_6$	C 53.84 H 5.23	53.50 5.52	387	387	1730 (s)

[a] Parent peak (P) represents the free base.

conformational free energy difference of the dimethylamino function is 2.4 kcal/mole (12). Therefore, if it is assumed that a methylene group between carbon 3 and the dimethylamino group lowers the conformational free energy difference [*e.g.*, $-\Delta G_x°$ values for $C(CH_3)_2$ and $CH_2C(CH_3)_3$ are 5.0 and 2.0 kcal/mole, respectively (12)], then at carbon 3 the phenyl ring will have the larger conformational free energy difference than the dimethylaminomethyl. Thus, the rotomer in which the C_6H_5–CH_3 interaction is minimal, *i.e.*, Rotomers 1 of III*a* and III*b*, predominate.

PMR spectroscopy (100 Hz) showed III*a* to have a coupling constant of 3.2 Hz between H_2 and H_3; hence a *gauche*-orientation is indicated between these hydrogens, and III*a* is considered to be the *threo*-isomer (13). Similarly, III*b*, with a coupling constant of 9.2 Hz, has a *trans*-coupling of hydrogens and is the *erythro*-isomer (13).

Examination of the mass spectra of the isomeric alcohols is consistent with the assignment of *threo*- and *erythro*-diastereoisomers to III*a* and III*b*, respectively, if the minor isomer (*erythro*) is more sterically crowded. The intensity of the molecular ion of the more crowded *erythro*-isomer would be predicted to be smaller than the less crowded *threo*-isomer (14), and the percent Σ_{25} values for the parent ions of III*a* and III*b* are 0.32 and 0.23%, respectively. The only significant fragment ion observed in the mass spectrum of either III*a* or III*b* is $CH_2{=}N^+(CH_3)_2$ (%Σ_{25} = 67.5%), *m/e* 58, formed by cleavage of the carbon–carbon bond next to the nitrogen (Scheme II).

Acylation of a mixture of III*a* and III*b* with either a substituted benzoyl chloride or phenoxyacetyl chloride afforded the corresponding ester series IV (Table I) and V in yields ranging from 20 to 83%. Examination of the PMR spectrum of V showed that the 1-methyl signals were downfield by approximately 1.7 ppm compared to IV*a*–IV*f*. For the phenoxyacetyl ester but not the benzoate esters, models revealed that it is possible for the phenoxyacetyl aromatic ring to assume a position perpendicular to the C-1 methyl group, which could account for the increased deshielding of the methyl protons. However, temperature-dependent studies did not alter the position of the 1-methyl signals, so alternative explanations for this phenomenon are required.

The evaluation of II–V against a wide range of microorganisms is shown in Table II. Mannich base II showed a promising level of activity against four fungi and two yeasts and had a spectrum of activity similar to the cyclic analog 2-dimethylaminomethyl-6-phenylcyclohexanone (7). The antimicrobial activity of II may be due to its structure *per se* or, alternatively, this β-aminoketone may deaminate, producing the corresponding α,β-unsaturated ketone (15, 16). Reduction of II led to abolition of antimicrobial activity in the screens chosen. Deamination of III*a*–III*b* to an α,β-unsaturated ketone would be unlikely, which may account for its inactivity, or the polar hydroxyl group may permit facile metabolism and detoxification to occur.

Masking of the polar hydroxyl groups produced the esters IV*a*–IV*f* and V. In series IV, there were different substituents in the acyl aromatic ring, but there appeared to be no correlation between the predicted hydrolysis rate and antimicrobial activity. Thus, while IV*b*, containing the electron-donating *p*-methoxy group, was four times as active as the unsubstituted ester, compounds containing the electron-withdrawing chlorine atoms were also more active than IV*a*. Furthermore, the nitro esters, IV*e* and IV*f*, for which the greatest hydrolysis would be predicted, were virtually devoid of antimicrobial activity. The nature of the substituents in the acyl aromatic ring in IV*a*–IV*f* seemed more important than the hydrolysis rate, and antimicrobial activity possibly was due to the esters *per se*. This possibility was strengthened by the observation that V was devoid of antifungal activity, suggesting that hydrolysis to phenoxyacetic acid did not occur.

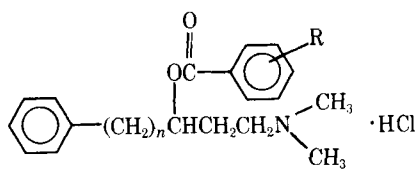
The antimicrobial results obtained parallel the results obtained for related series of Mannich bases and the corresponding alcohols and esters (7, 8). In general, the Mannich bases showed good levels of activity against fungi, while the corresponding alcohols were often bereft of antimicrobial potency. The esters containing chlorine as the nuclear acyl substituents were more active than esters whose nuclear substituents were methoxy or methyl; in turn, these esters displayed higher bioactivity than the unsubstituted esters, while nitro esters were almost invariably inactive.

In addition, in comparing the screening results of series VI and VII (R = H, CH_3, Cl, or NO_2), it was seen that a dimethylene chain (n = 2) between the phenyl ring and the carbon bearing the acyloxy function gave higher potency than when n = 0 (8). In series IV, in which one methylene group separated the aromatic ring from carbon 2, the average antimicrobial activity (8) was intermediate between series VI and VII.

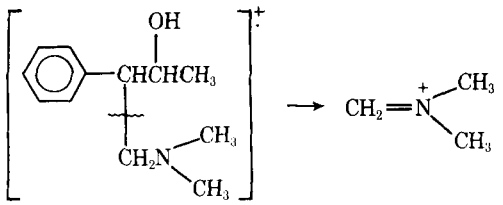
Both II and III*a* were evaluated in the L-1210 lymphoid leukemia screen in mice and shown to be toxic at 400 mg/kg in the Q4D and QD1-9 protocols, respectively (17). In the dose range of 12.5–200 mg/kg for II (QD1-9 protocol) and 100–200 mg/kg (QD1-9 protocol) for III*a*, in which cases murine toxicity was absent, the compounds were inactive. The alcohol III*a* was also screened against P-388 lymphocytic leukemia in mice in a dose range of 50–200 mg/kg (Q4D protocol) and shown to be nontoxic and inactive.

EXPERIMENTAL

Melting points are uncorrected. Organic extracts were washed with water and dried with anhydrous magnesium sulfate, and the solvent was removed using a water aspirator. Elemental analyses were performed[2]. Mass spectra[3] were determined at 70 ev. IR spectra[4] were determined



VI: n = 2
VII: n = 0



Scheme II

[2] R. M. Smith, College of Pharmacy, and R. E. Teed, Department of Chemistry and Chemical Engineering, University of Saskatchewan.

[3] AEI MS-12 mass spectrometer, Picker X-Ray Engineering Ltd., determined by D. R. Bain, Department of Chemistry and Chemical Engineering, University of Saskatchewan.

[4] Unicam SP 200 G spectrophotometer, Canlab Ltd., and IR 8 spectrophotometer, Beckman Instruments.

Table II—Screening of 4-Dimethylamino-3-phenyl-2-butanone Hydrochloride and Related Alcohols and Esters against Various Microorganisms[a]

Microorganisms[b]	II	III[c]	IV*a*[c]	IV*b*[d]	IV*c*[d]	IV*d*[e]	IV*e*[e]	IV*f*[d]	V[d]
Streptococcus faecalis	200	>200	>200	>200	>200	>200	>200	>200	>200
Staphylococcus aureus (*R*)[f]	200	>200	>200	>200	>200	>200	>200	>200	>200
Staphylococcus aureus (*S*)[g]	200	>200	>200	>200	>200	>200	>200	>200	>200
Klebsiella pneumoniae	200	>200	>200	>200	>200	>200	>200	>200	>200
Pseudomonas aeruginosa	>200	>200	>200	>200	>200	>200	>200	>200	>200
Escherichia coli	200	>200	>200	>200	>200	>200	>200	>200	>200
Salmonella typhimurium	200	>200	>200	>200	>200	>200	>200	>200	>200
Trichophyton mentagrophytes	<12	>200	200	200	50	50	>200	>200	>200
Mycobacterium smegmatis	50	>200	200	200	50	50	>200	200	>200
Candida albicans	200	>200	>200	>200	>200	200	>200	>200	>200
Bacillus subtilis	200	>200	>200	>200	200	200	>200	>200	>200
Fusarium oxysporum	<12	>200	>200	200	200	200	>200	>200	>200
Penicillium citrinium	<12	>200	>200	200	200	200	>200	>200	>200
Aspergillus niger	<12	>200	>200	200	200	200	>200	>200	>200
Cryptococcus neoformans	<12	>200	>200	200	200	200	>200	>200	>200
Blastomyces dermatitides	<12	>200	>200	200	200	200	>200	>200	>200
Xanthomonas vesicatoria	200	>200	>200	>200	200	200	>200	>200	>200
Streptococcus pyogenes	200	>200	>200	>200	>200	>200	>200	>200	>200
Sarcina lutea	200	>200	>200	>200	200	200	>200	>200	>200
Average antimicrobial activity[h]	584	0	11	37	84	89	0	6	0

[a] Figures in this table are the minimum inhibitory concentrations of the compounds in micrograms per milliliter. [b] The strains of microorganisms in this table are identified by the following numbers: ATCC 9790, SKF 24390, SKF 23390, SKF 4200, SKF 11320, SKF 12140, SKF 11350, SKF 17410, ATCC 101, SKF 3470, ATCC 6633, ATCC 9848, ATCC 16040, SKF 330, EK1, EK2, ATCC 11551, ATCC 8668, and ATCC 9341, respectively. [c] Screened as a mixture of equal amounts of *threo*- and *erythro*-isomers. [d] Pure *erythro*-isomer. [e] Configuration of screening sample not determined. [f] Strain resistant to penicillin G. [g] Strain sensitive to penicillin G. [h] Figures were evaluated from the following expression: (combined antimicrobial activity × 100)/number of microorganisms in screen. The combined antimicrobial activity was determined by giving the following scores at the highest potency of the compound against the microorganism: 200 μg = 1, 50 μg = 4, and <12 μg = 16.

as potassium bromide disks unless otherwise stated. NMR spectra[5] (60 Hz) were carried out in deuterochloroform using tetramethylsilane as the internal standard unless otherwise stated. The 100-Hz NMR spectra[6] also were determined.

4-Dimethylamino-3-phenyl-2-butanone Hydrochloride (II)—A mixture of phenylacetone (22.4 g, 0.167 mole), paraformaldehyde (8.0 g, 0.265 mole), dimethylamine hydrochloride (21.6 g, 0.265 mole), hydrochloric acid (1 ml), and ethanol (100 ml) was heated under reflux with stirring for 4 hr. On cooling, the solvent was removed to give a pale-yellow oil. This oil solidified on trituration with dry ether, yielding a pale-yellow powder (32 g). Recrystallization of the solid from 2-propanol gave II as colorless cubes (23.3 g, 61%), mp 145–146° [lit. (18) mp 145–146°]; NMR (D_2O): δ 2.15 (s, 3H, CH_3), 2.95 [s, 6H, $N(CH_3)_2$], 3.70 (m, 2H, CH_2), 4.55 (t, 1H, CH), and 7.50 (m, 5H, C_6H_5) ppm.

Anal.—Calc. for $C_{12}H_{18}ClNO$: C, 63.29; H, 7.97. Found: C, 63.00; H, 8.12.

Reduction of 4-Dimethylamino-3-phenyl-2-butanone with Lithium Aluminum Hydride—4-Dimethylamino-3-phenyl-2-butanone (94.4 g, 0.493 mole) in dry ether (200 ml) was added dropwise to a stirring suspension of lithium aluminum hydride (9.40 g, 0.247 mole) in dry ether (300 ml). The reaction mixture was heated under reflux for 4 hr, cooled, and decomposed by the dropwise addition of water (25 ml). The inorganic material was removed by filtration, and a pale-yellow syrup consisting of a mixture of *erythro*- and *threo*-4-dimethylamino-1-phenyl-2-butanols (76.7 g, 81%) was obtained from the organic extract; IR (smear); 3400 s (OH) and 1705 s (C=O) cm^{-1} (absent).

The NMR (CD_2Cl_2) spectrum showed that the ratio of *erythro*- and *threo*-isomers was 40:60 by integration of the methyl doublets. A small quantity of the reaction mixture (5.0 g) was dissolved in dry acetone (25 ml) and acidified with ethanolic hydrochloric acid (20% v/v). The colorless crystals (4.1 g) that deposited were fractionally crystallized from dry acetone to give III*a* (1.8 g) as colorless needles, mp 142–143°; NMR (CD_2Cl_2): δ 7.3 (m, 5H, C_6H_5), 4.35 (m, 1H, C-2 H), 3.9 (m, 1H, C-4 H), 3.2 (m, 2H, C-4 H, C-3 H), 2.36 [s, 6H, $N(CH_3)_2$], and 0.90 (d, 3H, C-1 H_3) ppm.

Anal.—Calc. for $C_{12}H_{20}ClNO$: C, 62.73; H, 8.77; N, 6.10. Found: C, 62.75; H, 8.80; N, 6.12.

From the acetone mother liquor, the second isomer was obtained. It was recrystallized from dry acetone to give III*b* (1.6 g) as colorless shiny plates, mp 93°; NMR (CD_2Cl_2): δ 7.3 (m, 5H, C_6H_5), 4.0 (m, 1H, C-2 H), 3.8 (m, 1H, C-4 H), 3.2 (m, 1H, C-4 H), 3.1 (m, 1H, C-3 H), 2.32 [s, 6H, $N(CH_3)_2$], and 1.05 (d, 3H, C-1 H_3) ppm.

Anal.—Calc. for $C_{12}H_{20}ClNO$: C, 62.73; H, 8.77. Found: C, 62.60; H, 8.98.

Esters of 4-Dimethylamino-3-phenyl-2-butanol (IV*a*–IV*f*)—These compounds were prepared by the following general method. A solution of the acid chloride (0.025 mole) in dry ether (35 ml) was added dropwise to a stirring solution of 4-dimethylamino-3-phenyl-2-butanol (crude reaction mixture, 0.025 mole) in dry ether (35 ml). The temperature of the reaction mixture was maintained at 0–5° during addition, and then the solution was stirred at room temperature for at least 8 hr. The precipitate was removed by filtration and recrystallized from ether–ethanol to give colorless crystals, except in the case of the nitro esters which were pale yellow in color.

The physical data of the esters are summarized in Table I. NMR spectra of available esters indicated that IV*b*, IV*c*, and IV*f* consisted of only the *erythro*-isomer ($JH_{2,3}$ = 8.0, 8.1, and 6.9 Hz, respectively) while IV*a* consisted of a 50:50 mixture of the *erythro* ($JH_{2,3}$ = 8.0 Hz) and *threo* ($JH_{2,3}$ = 2.4 Hz) isomers.

4-Dimethylamino-3-phenyl-2-butanol Phenoxyacetate Hydrochloride (V)—The general esterification described earlier was employed, except that dry benzene was used as the solvent. The desired ester hydrochloride (5%) was obtained as colorless prisms, mp 170–172°; mass spectrum: 327 (M^+ − HCl).

Anal.—Calc. for $C_{20}H_{26}ClNO_3$: C, 66.01; H, 7.20. Found: C, 65.50; H, 7.47.

Screening of Compounds—The antimicrobial evaluations were undertaken according to the published procedure[7] (7, 8). The antineoplastic screens were accomplished using the reported protocols (17).

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[5] WP 60 spectrophotometer, Brucker Spectrospin (Canada) Ltd., and T60 spectrophotometer, Varian Associates of Canada Ltd.

[6] HA 100-12 spectrophotometer, Varian Associates of Canada Ltd. Determined in the Chemistry Department, University of Alberta.

[7] Carried out by Dr. J. F. Pagano and staff at Smith Kline and French Laboratories, Philadelphia, PA 16101.

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Simple High-Pressure Liquid Chromatographic Determination of Trisulfapyrimidines in Human Serum

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Abstract □ A simple and rapid high-pressure liquid chromatographic method was developed for the determination of sulfadiazine, sulfamerazine, and sulfamethazine in human serum. After the trichloroacetic acid precipitation of the serum proteins, an aliquot of the supernate is injected into a high-pressure liquid chromatograph equipped with a reversed-phase microparticulate column and a fixed wavelength UV detector. For each of the three components of trisulfapyrimidines, a linear calibration curve was observed in the 1–30-μg/ml range, with the precision of the assay estimated to be ±2% (*RSD*). Preliminary pharmacokinetic data are also presented.

Keyphrases □ Sulfadiazine—high-pressure liquid chromatographic analysis in human serum □ Sulfamethazine—high-pressure liquid chromatographic analysis in human serum □ Sulfamerazine—high-pressure liquid chromatographic analysis in human serum □ High-pressure liquid chromatography—analyses, sulfadiazine, sulfamethazine, and sulfamerazine in human serum □ Antibacterials—sulfadiazine, sulfamethazine, and sulfamerazine, high-pressure liquid chromatographic analyses in human serum □ Trisulfapyrimidines—high-pressure liquid chromatographic analyses in human serum

The antibacterial properties of the sulfonamides were first recognized with the discovery of sulfamidochrysoidine (1), and these drugs are still frequently used in the treatment of urinary tract infections. Unfortunately, kidney damage has been associated with sulfonamide therapy, resulting from the crystallization in the renal tubules of the N^4-acetylated metabolites of many sulfonamides. To overcome this problem, combinations of sulfa drugs are commonly utilized. One such combination is the trisulfapyrimidines: sulfadiazine, sulfamerazine, and sulfamethazine.

A colorimetric method based on a diazotization reaction, as proposed by Bratton and Marshall (2), is commonly employed for quantitating sulfonamides. However, when applied to biological fluids, interferences from metabolites possessing a free amino group was observed. To overcome this lack of specificity, Rieder (3) introduced an extraction step.

Many other spectrophotometric and TLC methods subsequently were proposed for the determination of sulfonamides in biological fluids (4–12). GLC of the sulfa drugs also was attempted (13–15). However, derivatization must be performed to eliminate adsorption of these compounds to the chromatographic support. The separation and quantitation of sulfa drugs, alone (16–18) and in pharmaceutical preparations (19, 20), by high-pressure liquid chromatography (HPLC) were described. Recently, methods were proposed for sulfamethazine residues in bovine tissue (21) and for sulfamethazine, sulfamerazine, sulfathiazole, and their metabolites in cattle urine (22).

A study relating dissolution and bioavailability profiles of triple sulfa suspensions required a specific and sensitive method for the individual trisulfapyrimidine components. This report describes a simple, rapid, specific, and sensitive HPLC method devised for this purpose.

EXPERIMENTAL

Apparatus—A modular high-pressure liquid chromatograph consisted of a constant-flow pump[1], a valve-type injector[2], a fixed wavelength (254 nm) UV detector[3], and a strip-chart recorder[4]. A stainless steel column (3.9 mm × 30 cm) packed with fully porous 10-μm silica particles, to which is chemically bonded a monomolecular layer of octadecylsilane[5], was obtained commercially.

Chromatographic Conditions—The mobile phase consisted of acetonitrile–1% acetic acid (13:87). A flow rate of 1.5 ml/min was established (1100 psi). The column was maintained at 29.6° by inserting it into a glass sleeve which was then immersed in a constant-temperature water bath[6].

[1] Chromatography pump model M-6000A, Serial No. SDS-5235, Waters Associates, Milford, Mass.
[2] Universal injector model U6K, Serial No. U6K-6065, large capacity, Waters Associates, Milford, Mass.
[3] Model 440 absorbance detector, Serial No. 440-01249, Waters Associates, Milford, Mass.
[4] A-25 dual channel, Varian Associates, Walnut Creek, Calif.
[5] Waters Associates prepacked μBondapak C_{18} column.
[6] B. Braun Thermomix II (No. 26394), Bronwill Scientific Co., Rochester, N.Y.