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Partial Synthesis of Harringtonine Analogs

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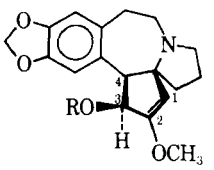
Abstract □ Three analogs of harringtonine (II), an ester alkaloid which is active in the P-388 experimental leukemia system, were prepared by acylating cephalotaxine (I). They were the 2-hydroxy-2-methylbutyryl (III), 2-carbomethoxymethyl-5-methylhexanoyl (IX*c*), and 2-carbomethoxymethylene-5-methylhexanoyl (VII*d*) esters of I. A special sequence was developed for the synthesis of III. All of these harringtonine analogs, with the possible exception of III, are inactive in the P-388 system. In addition, data are presented which show that a rearranged harringtonine isomer (X) also is inactive. These results emphasize some highly specific structural requirements for antitumor activity of *Cephalotaxus* alkaloids.

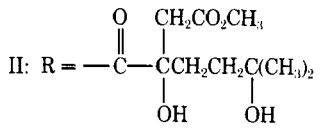
Keyphrases □ Harringtonine alkaloid analogs—partial synthesis, screened for antitumor activity □ *Cephalotaxus harringtonia* alkaloid analogs—partial synthesis, screened for antitumor activity □ Cephalotaxine esters—partial synthesis □ Antitumor agents, potential—partial synthesis of harringtonine analogs

Cephalotaxus harringtonia plant materials contain cephalotaxine (I) as well as esters of this alkaloid incorporating various dicarboxylic acids. Harringtonine (II) and certain other naturally occurring esters of cephalotaxine exhibit significant antitumor activity (1, 2). Because of its novel chemical structure and the pharmacological activity of its esters, the total synthesis of I was undertaken by at least three groups (3–5), two of which reported successful syntheses in preliminary form (3, 4). Cephalotaxine, although inactive, is by far the most abundant of the *Cephalotaxus* alkaloids; the active ester alkaloids needed for clinical testing remain in short supply.

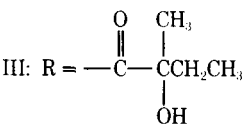
The active alkaloids are not readily prepared by direct esterification of cephalotaxine because of marked steric hindrance at the reaction site in both the acid and alkaloid moieties. An α-hydroxy group in the acid moiety further complicates synthetic efforts. A sequence was developed for the preparation of a harringtonine analog (III) whose acid moiety (2-hydroxy-2-methylbutyric acid, V*a*) incorporates some structural features found in the active compounds. The purpose of the present study was to develop synthetic procedures by which active *Cephalotaxus* alkaloids could be prepared from cephalotaxine as well as to ascertain which structural features are essential for the antitumor activity of this group of alkaloids.



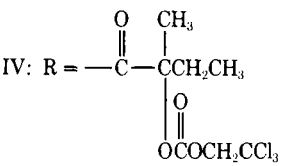
I: R = H



II: R = —C(=O)—CH(CH$_2$CO$_2$CH$_3$)—CH$_2$CH$_2$C(CH$_3$)$_2$(OH)—OH



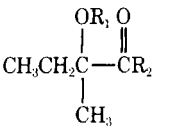
III: R = —C(=O)—CH(CH$_3$)—CH$_2$CH$_3$ (OH)



IV: R = —C(=O)—CH(CH$_3$)—CH$_2$CH$_3$ (O—C(=O)—CH$_2$CCl$_3$)

DISCUSSION

The first attempt to prepare 2-hydroxy-2-methylbutyrylcephalotaxine (III) was made with the α-hydroxy group blocked by a benzyl ether linkage. Treatment of cephalotaxine with the blocked acid chloride provided the desired ester, but the benzyl group could not be removed by hydrogenolysis. Therefore, the sequence described here was employed to solve the problem of protecting the hydroxy group. It is anticipated that this route will be useful in the preparation of other α-hydroxy esters of cephalotaxine.



V*a*: R_1 = H, R_2 = OH

V*b*: R_1 = H, R_2 = —OCH$_2$C$_6$H$_5$

V*c*: R_1 = —C(=O)—CH$_2$CCl$_3$, R_2 = —OCH$_2$C$_6$H$_5$

V*d*: R_1 = —C(=O)—CH$_2$CCl$_3$, R_2 = OH

V*e*: R_1 = —C(=O)—CH$_2$CCl$_3$, R_2 = Cl

Although the benzyl ester (V*b*) and its derivative (V*c*) with the hydroxyl blocked by a trichloroethoxycarbonyl group are prepared readily, the hydrogenolysis and subsequent formation of the acid chloride (V*e*) occurred at a slower rate than expected. The trichloroethoxycarbonyl blocking group was readily removed from IV with zinc–acetic acid (6) after acylation of cephalotaxine.

The racemic form of the deoxyharringtonine dicarboxylic acid moiety (VI*a*), synthesized previously (2), was dehydrated (as its dimethyl ester) with thionyl chloride to provide two isomeric dehydration products, VII*a* and VIII*a*. These compounds were readily separated chromatographically and were distinguished by NMR spectra; the olefinic proton appeared in the spectrum of VII*a* as a singlet at δ 6.70, whereas the corresponding signal for VIII*b* was a triplet at δ 7.13. Hydrogenation of a mixture of VII*a* and VIII*a* afforded IX*a*. Diesters VII*a* and IX*a* were converted to the requisite half-esters by the partial esterification sequence developed previously (2) and were used to acylate cephalotaxine *via* the corresponding acid chlorides.

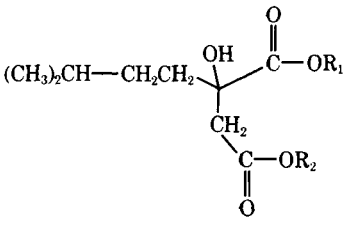
The alkaloid esters prepared were tested[1] for antitumor activity in the P-388 lymphocytic leukemia system. As shown in Table I, no activity was detected. Alkaloid ester X was prepared by partial synthesis previously[2], but test data have just become available. One test, at 20 mg/kg, indicated marginal activity, but the figure may not be reliable since tests at both higher and lower dose levels showed no activity. Alkaloid derivative III is an allylic ester. It has a hindered tertiary carboxyl group and an α-hydroxy group. Even though III incorporates these three features of the active natural cephalotaxine esters, its activity, if any, is of a much lower order of magnitude than that of the natural esters. This result exemplifies the high degree of structural specificity often required of antitumor compounds. However, some structural variation in the acyl moiety is allowed since homoharringtonine, isoharringtonine, and deoxyharringtonine, as well as harringtonine itself, all exhibit considerable antitumor activity (1, 2).

EXPERIMENTAL[3]

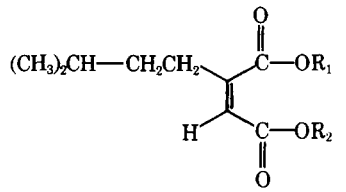
Hydroxy-Blocked Acid Chloride (V*e*)—Racemic V*a* (5.90 g), 5.00 ml of benzyl alcohol, and 0.080 g of *p*-toluenesulfonic acid catalyst were dissolved in 50 ml of benzene. Water formed during the 6-hr reflux period and was removed by azeotropic distillation with a Dean–Starke tube. The benzene solution was washed (2 × 10 ml) with 5% aqueous sodium bicarbonate and with water (3 × 10 ml) and was dried over anhydrous magnesium sulfate. Evaporation of benzene to constant weight gave V*b*. This benzyl ester (9.10 g) in 10 ml of dry chloroform was treated with 20 ml of pyridine and cooled to 0°. Then 1.10 equivalents of β,β,β-trichloroethoxycarbonyl chloride (6) in 20 ml of chloroform was added and the solution was stirred at 0° for 1 hr and then at 25° overnight. After most of the chloroform was evaporated, 100 ml of ether was added and the mixture was acidified with cold dilute hydrochloric acid. Product V*c* was recovered by ether extraction; NMR: δ 0.91 (t, J = 7.5 Hz, 3H, terminal —CH_3), 1.64 (s, 3H, branched —CH_3), 1.96 (m, at least eight lines visible, 2H, —CH_2CH_3), 4.66 (s, 2H, —CH_2CCl_3), 5.16 (s, 2H, —$CH_2C_6H_5$), and 7.33 (s, 5H, aromatic). The mass spectrum of V*c* showed no molecular ion but exhibited prominent ions at *m/e* 190 (10), 131 (15), 91 (100), 84 (78), and 63 (71).

Anal.—Calc. for $C_{15}H_{17}Cl_3O_5$: C, 47.12; H, 4.46; Cl, 27.58. Found: C, 47.52; H, 4.57; Cl, 28.11.

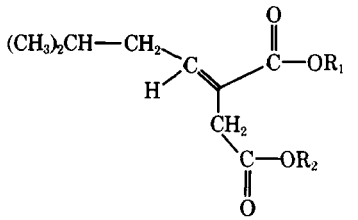
Hydrogenolysis of 5.70 g of V*c* dissolved in 15 ml of ethanol containing 0.30 g of 10% palladium-on-carbon for 4 hr at ambient conditions yielded V*d* as shown by the absence of the methylene singlet, δ 5.16, and the aromatic proton singlet, δ 7.33, from its NMR spectrum. Acid V*d* (3.85 g) was converted to its acid chloride V*e* by stirring for 65 hr at room temperature with 10 ml neat oxalyl chloride and then removing excess reagent under vacuum at room temperature. Products V*b*, V*c*, V*d*, and V*e* were recovered in essentially quantitative yields.



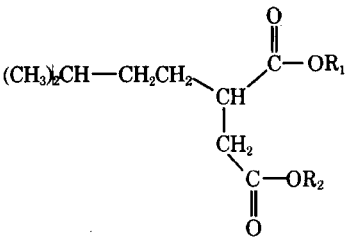
VI*a*: $R_1 = R_2 = H$
VI*b*: $R_1 = R_2 = CH_3$



VII*a*: $R_1 = R_2 = CH_3$
VII*b*: $R_1 = R_2 = H$
VII*c*: $R_1 = H$, $R_2 = CH_3$
VII*d*: R_1 = cephalotaxine moiety, $R_2 = CH_3$



VIII*a*: $R_1 = R_2 = CH_3$
VIII*b*: $R_1 = H$, $R_2 = CH_3$



IX*a*: $R_1 = R_2 = CH_3$
IX*b*: $R_1 = H$, $R_2 = CH_3$
IX*c*: R_1 = cephalotaxine moiety, $R_2 = CH_3$

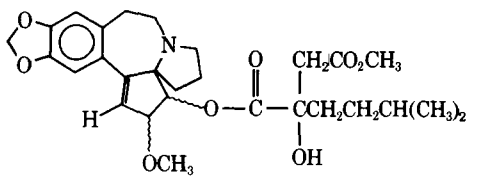
2-Hydroxy-2-methylbutyrylcephalotaxine (III)—Cephalotaxine (I), 2.60 g in 10 ml of pyridine was treated with small portions of a cold solution of 4.00 g of V*e*, also in pyridine (5 ml), and stirred at room temperature for 6 days [acid chloride–cephalotaxine (1.5:1)]. The solution was basified with 5 ml of 5% aqueous sodium carbonate solution and extracted with chloroform. Analytical TLC with methanol–chloroform (15:85) indicated the major product (IV) had R_f 0.58. Purification of IV by preparative TLC gave a yield of 40%; NMR: δ 0.62 and 0.66 (2 overlapping t[4], J = 7.5 Hz, 3H, terminal —CH_3), 1.24 and 1.27 (2s, 3H, branched —CH_3), 1.3–3.4 (overlapping m, methylene protons), 3.66 and 3.67 (2s, 3H, —OCH_3), 3.78 (d, J = 9 Hz, 1H, C-4 proton), 4.68 (s, 2H, —CH_2CCl_3), 5.04 (s, fine splitting, J < 1 Hz, 1H, vinyl), 5.84 (s, 2H,

[1] Assays were performed under Drug Research and Development auspices. Procedures are described in *Cancer Chemother. Rep.*, **25**, 1(1962).

[2] K. L. Mikolajczak, C. R. Smith, and R. G. Powell, unpublished results.

[3] NMR spectra were determined on $CDCl_3$ solutions with a Varian HA-100 instrument, and IR spectral analyses were done on 1% chloroform solutions with a Perkin-Elmer model 137 spectrometer. Low-resolution mass spectral data were determined with a Du Pont CEC 21-492-1 spectrometer, and high-resolution spectra were determined with a Nuclide 12-90 G spectrometer. Analytical TLC was done on Brinkmann silica gel F-254, 0.25-mm, precoated plates. Spots were visualized by observing the plates under UV light and by staining with iodide vapor. Preparative separations were achieved with 1-mm silica gel G plates developed with 7% methanol in chloroform. Spots were visualized with 0.5% bromthymol blue in ethanol solution.

[4] Since the α-hydroxy acid V*a* is racemic, the derived esters III and IV are mixtures of diastereomers; therefore, the chemical shifts of NMR signals associated with certain protons may be different for each isomer.



X

—OCH_2O—), 5.87 and 5.91 (2d, J = 9 Hz, fine splitting, J < 1 Hz, 1H, C-3 proton), and 6.59 (broadened s, 2H, aromatic). The mass spectrum of IV gave ions at m/e 589 (M^+, 6%), 314 (10), 298 (100), 271 (19), and 150 (17).

Anal.—Calc. for $C_{26}H_{30}Cl_3NO_8$: C, 52.98; H, 5.09; Cl, 17.82; N, 2.37. Found: C, 52.44; H, 5.21; Cl, 18.18; N, 2.24.

Ester IV (4.68 g) was stirred with an equal weight of activated zinc dust[5] in 11 ml of acetic acid for 48 hr at room temperature. This solution was diluted with 75 ml of ether and was washed with 5% aqueous sodium bicarbonate until the wash remained basic. The yield of III by preparative TLC was 60% of theoretical; IR: 3600 (—OH) and 1725 (—C=O) cm^{-1}; NMR: δ 0.40 and 0.75 ($2t^4$, J = 7.5 Hz, 3H, terminal —CH_3), 0.72 and 1.15 ($2s^4$, 3H, branched —CH_3), 1.3–3.3 (overlapping m, methylene protons), 3.68 (s, 3H, —OCH_3), 3.78 (d, J = 9 Hz, 1H, C-4 proton), 5.06 (s, 1H, vinyl), 5.85 (s, 2H, —OCH_2O—), 5.90 and 5.93 (2d, J = 9 Hz, 1H, C-3 proton), and 6.58, 6.59, and 6.62 [3s, 2H, aromatic (2s from one diastereomer and 1s from the other)]. The mass spectrum of III exhibited prominent ions at m/e 415 (M^+, 43%), 389 (13), 314 (21), 298 (100), 266 (17), and 150 (22).

Anal.—Calc. for $C_{23}H_{29}NO_6$: C, 66.50; H, 6.99; N, 3.37. Found: C, 66.01; H, 7.28; N, 3.44.

Methyl 2-Carbomethoxymethylene-5-methylhexanoate (VII*a*)—Compound VI*b* (2.40 g), prepared as described previously (2), was dissolved in 2.0 ml of pyridine at 0°; 1.90 ml of thionyl chloride was added and the mixture was heated under reflux 1 hr. Ice was added and the resulting mixture was extracted repeatedly with ether. The combined ether extracts were washed with aqueous sodium carbonate and then with water and were dried over anhydrous magnesium sulfate. Ether was evaporated to provide 2.09 g of the crude dehydration product. Analytical TLC of this mixture with hexane–ether–acetic acid (50:50:2) as the developing solvent revealed two spots; its IR spectrum showed no hydroxy absorption. The two components were separated by column chromatography; 1-g portions of the mixture were applied to a 2.4 × 30-cm column[6] and eluted successively with 100 ml of hexane, 200 ml of 5% ether in hexane, 200 ml of 10% ether in hexane, and finally 200 ml of 30% ether in hexane. The more mobile component (VII*a*) was a liquid representing 55% of the total product recovered; NMR: δ 0.91 (d, 6H, J = 6 Hz, isopropyl methyls), 1.2–1.5 (m, 1H at C-5), 1.38 (distorted t[7], 2H, J = 8 Hz, C-3 methylene protons), 2.78 (distorted t[7], 2H, J = 8 Hz, C-4 methylene protons), 3.73 and 3.77 (2s, 3H each, carbomethoxy protons), and 6.70 (s, 1H, olefinic proton). Mass spectroscopy showed: $M^+ - 15$, m/e 199.094; $C_{11}H_{18}O_4 - CH_3$ requires 199.097, and $M^+ - 31$, m/e 183.100; $C_{11}H_{18}O_4 - CH_3O$ requires 183.102.

The less mobile diester (VIII*a*), 45% of the dehydrated material resolved by column chromatography, was obtained as a liquid. Mass spectroscopy showed: $M^+ - 15$, m/e 199.094; $C_{11}H_{18}O_4 - CH_3$ requires 199.097, and $M^+ - 31$, m/e 183.100; $C_{11}H_{18}O_4 - CH_3O$ requires 183.102. Spectroscopic characterization of the corresponding half-ester (VIII*b*) is given in the next section.

2-Carbomethoxymethylene-5-methylhexanoic Acid (VII*c*)—Compound VII*a* (1.39 g) was stirred with 1 *N* sodium hydroxide in methanol–water (40:60) for 3 days at ambient temperature. The mixture was then acidified with dilute hydrochloric acid and extracted repeatedly with ether; the combined extracts were dried with anhydrous magnesium sulfate and evaporated to provide as an amorphous solid, 0.94 g of dicarboxylic acid VII*b*.

Anal.—Calc. for $C_9H_{14}O_4$: C, 58.16; H, 7.53. Found: C, 57.97; H, 7.41.

[5] Activated zinc was prepared by washing zinc dust for 10 min with 2 *N* HCl, then five times with water, and then three times with methanol before drying at 110°.

[6] Adsorbosil, Applied Science Laboratories.

[7] These two triplets, distorted by second-order splitting, are mirror images that represent a typical A_2X_2 system (7).

Table I—Test Data for Synthetic Analogs of Harringtonine in P-388 Lymphocytic Leukemia System[a]

Alkaloid	Dose, mg/kg	Survivors	Animal Weight Difference (T − C)	Survival Time, Days (T/C)	T/C, %
III	135	4/4	−1.7	13.5/10.0	135
	40	6/6	1.6	10.5/10.0	105
	10	6/6	0.5	9.0/10.0	90
	2.5	6/6	1.8	9.5/10.0	95
VII*d*	65	4/4	−1.7	12.5/11.0	113
	45	4/4	−0.3	13.0/11.0	118
	20	4/4	−0.7	10.5/11.0	95
	5	4/4	−2.2	11.5/11.0	104
IX*c*	40	6/6	0.1	11.5/11.0	104
	20	6/6	−1.8	13.0/11.0	118
	5	6/6	0.2	11.5/11.0	104
X	29	4/4	−0.3	11.5/10.0	115
	20	4/4	−1.5	14.0/11.0	127
	10	4/4	−0.2	11.0/11.0	100
	5	4/4	−0.3	11.0/11.0	100

[a] Materials were considered active if the survival time of animals treated (T) with them was ≥ 125% of that of the controls (C) (*i.e.*, T/C ≥ 125%).

This diacid was converted to the requisite half-ester (VII*c*), a viscous liquid, by treatment with dioxane–methanol–sulfuric acid, essentially as described previously for 3-carboxy-3-hydroxy-6-methylheptanoic acid (2).

Ester VIII*a* was similarly converted to the corresponding half-ester, 2-carbomethoxymethyl-5-methyl-2-hexenoic acid (VIII*b*), a viscous liquid; NMR: δ 0.92 (d, 6H, J = 6 Hz, isopropyl methyls), 1.2–2.0 (m, 1H, methine proton at C-5), 2.10 (overlapping dd, 2H, J = 7 Hz for each, C-4 methylene protons), 3.34 (s, 2H, methylene in side chain), 3.66 (s, 3H, carbomethoxy protons), and 7.13 (t, 1H, J = 7 Hz, olefinic proton at C-3).

2-Carbomethoxymethyl-5-methylhexanoic Acid (IX*b*)—The dehydration product from VI*a* was converted to a mixture of half-esters *via* the hydrolysis–esterification sequence described for VII*a*. A 1.20-g portion of the resulting product (VII*c* + VIII*b*) was hydrogenated in methanol with 10% palladium-on-carbon catalyst (ambient temperature, pressure). After the catalyst was removed by filtration, solvent was evaporated to afford the saturated half-ester IX*b*, a liquid; NMR: δ 0.86 (d, 6H, J = 6 Hz, isopropyl methyls), 1.0–1.8 (m, 1H, methine proton at C-5), 1.28 (td, 2H, $J_{3,4}$ = 6 Hz, $J_{4,5}$ = 0.5 Hz, C-4 methylene protons), 1.53 (dt, 2H, $J_{2,3}$ = 12 Hz, $J_{3,4}$ = 6 Hz, C-3 methylene protons), 2.2–3.0 (m, 1H, methine proton at C-2), 2.63 (dd, 2H, J = 13 Hz, side-chain methylene), and 3.63 (s, 3H, carbomethoxy protons). The mass spectrum of IX*a* exhibited ions at m/e 185 (29), 146 (60), 143 (42), and 114 (100). Found: $M^+ - 31$, m/e 185.116; $C_{11}H_{20}O_4 - OCH_3$ requires 185.118.

2 - Carbomethoxymethylene - 5 - methylhexanoylcephalotaxine (VII*d*)—Half-ester VII*c* (0.37 g) was treated for 2 hr with 2 ml of oxalyl chloride containing 1 drop of pyridine. After evaporation of solvents *in vacuo*, the acid chloride was stirred at room temperature with 0.30 g of cephalotaxine in 2 ml of pyridine for 72 hr. The resulting ester (VII*d*) was purified by preparative TLC yielding 57%; NMR: δ 0.84 and 0.87 (2d, 6H total, J = 6 Hz, isopropyl methyls), 1.1–3.2 (overlapping m), 3.67 (s, 6H, vinyl methoxy and carbomethoxy), 3.78 (d, 1H, J = 10 Hz, proton on C-4), 5.05 (s, 1H, vinyl of cephalotaxine moiety), 5.80 (s, 2H, methylenedioxy), 5.88 (d, 1H, J = 10 Hz, proton on C-3), 6.22 (s, 1H, vinyl of acyl moiety), and 6.51 and 6.53 (2s, 1H each, aromatic). Mass spectral analysis of VII*d* gave ions at m/e 497 (M^+, 34), 466 (11), 314 (48), 298 (100), 282 (8), 266 (18), 228 (4), 214 (6), 150 (16), and 137 (10).

Anal—Calc. for $C_{28}H_{35}NO_7$: C, 67.61; H, 7.04; N, 2.81. Found: C, 67.37; H, 7.11; N, 2.73.

2-Carbomethoxymethyl - 5 - methylhexanoylcephalotaxine (IX*c*)—Half-ester IX*b* (1.00 g) was treated with oxalyl chloride for 1 hr at room temperature, and excess reagent was evaporated *in vacuo*. The resulting acid chloride (1.27 g) in 3 ml of pyridine was added to 0.875 g of I in 10 ml of pyridine. The solution was heated at 50° for 3 hr and then stirred at room temperature for 3 days. The yield of IX*c* from preparative TLC was 81%; NMR: δ 0.78 and 0.83 (2d, 6H total, J = 6 Hz, isopropyl methyls), 1.1–3.2 (overlap-

ping m, methylenes and methines), 3.59 (s, 3H, vinyl methoxy), 3.64 and 3.66 (2s, 3H total, carbomethoxy), 3.72 (d, 1H, J = about 10 Hz, benzylic proton on C-4), 5.00 (s, 1H, vinyl), 5.82 (s and an indistinct overlapping signal, 2H plus 1H, methylenedioxy and proton on C-3), and 6.64 and 6.67 (2s, 1H each, aromatic). Its mass spectrum showed significant ions at m/e 499 (M^+, 40), 469 (12), 314 (19), 298 (100), 266 (17), 185 (36), 173 (29), 146 (71), and 114 (78); high-resolution M^+: calc. for $C_{28}H_{37}NO_7$, 499.257; found, 499.257.

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References to specific equipment or commercial firms are made for clarity and do not necessarily constitute endorsement by the U.S. Department of Agriculture over other products or firms not mentioned.

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GLC Determination of Theophylline in Biological Fluids

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Abstract □ A specific GLC procedure for the determination of theophylline in plasma and saliva is described. The method selectively measures theophylline in the presence of its metabolites and other xanthines. The GLC method is sufficiently sensitive to detect 1 μg theophylline/ml plasma or saliva. Analysis for theophylline in plasma and saliva samples obtained from patients showed a saliva–plasma ratio of approximately 0.5.

Keyphrases □ Theophylline—GLC determination in biological fluids in presence of metabolites and other xanthines □ Xanthine derivatives—GLC determination of theophylline in biological fluids in presence of metabolites and other xanthines □ GLC—determination, theophylline in biological fluids

A specific and sensitive analytical method for the quantitative determination of a drug in blood and urine is needed to follow the kinetics of the substance in the body and to control the method of administration to secure optimal effects. Theophylline (1,3-dimethylxanthine) is a widely used xanthine for its antiasthmatic and cardiovascular actions. The method of Truitt *et al.* (1) for the determination of theophylline was based upon spectrophotometric measurement of the azo dye developed by coupling it with Fast Blue 2B. The method of Plummer (2) involved converting theophylline into a copper complex, mixing with potassium iodide solution, and titrating the liberated iodine with thiosulfate. Both these methods are tedious and require large quantities of blood. A UV spectrophotometric method (3–5) is more frequently used for the determination of theophylline in plasma. The sensitivity of this method is good but is subject to interference from other xanthines present in plasma, namely caffeine, theobromine, and theophylline metabolites. In addition, endogenous compounds such as xanthine, hypoxanthine, and uric acid contribute to the absorbance blank. Barbiturates, if present, also interfere in the absorption measurements of theophylline. The presence of these compounds results in an overestimation of the theophylline concentration in the sample. Gupta and Lundberg (6) described a differential spectrophotometric method for determination of theophylline in the presence of barbiturates.

A fast, sensitive, and selective GLC method for the determination of theophylline in plasma and saliva is reported here. It can be used in the presence of theophylline metabolites, caffeine, theobromine, or any barbiturates. If needed, the modified plasma extraction procedure can be used for spectrophotometric analysis.

EXPERIMENTAL

Materials—Analytical grade solvents and chemicals were used without further purification.

Apparatus—A chromatograph[1] equipped with a flame-ionization detector and a 1.83-m × 0.31-cm (6-ft × 0.125-in.) glass column packed with 3% OV-17 on Gas Chrom Q was used. The following conditions of the chromatograph were satisfactory for the separation of theophylline and its metabolites and the internal standard from the extracted components of plasma: injector port temperature, 265°; column temperature, 190°; detector block temperature, 280°; and nitrogen flow, 35 ml/min. Hydrogen and air flow were optimized for maximum response. The retention times

[1] Varian Aerograph model 1200.