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RESEARCH ARTICLES

Controlled Delivery of Theophylline: Chemistry of 7-Acyl- and 7,7′-Acylditheophylline Derivatives

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Abstract □ 7-Acyl- and 7,7′-acyldithophylline derivatives were prepared from the reaction of theophylline with acid chlorides. In addition, a novel synthesis of these compounds was developed, which proceeds through an acylonium ion generated under mild conditions. The physical properties and stability of the derivative of choice, 7,7′-succinyldithophylline, depend on the synthetic procedure employed. This compound is a useful controlled-release prodrug of theophylline.

Keyphrases □ Theophylline—various 7-acyl- and 7,7′-acyldithophylline derivatives synthesized, evaluated as prodrugs □ Prodrugs, potential—7,7′-succinyldithophylline synthesized, evaluated □ Relaxants, smooth muscle—theophylline, various 7-acyl- and 7,7′-acyldithophylline derivatives synthesized, evaluated as prodrugs

One widely used treatment for asthma is oral theophylline. Where long-term treatment of asthma is indicated, theophylline therapy has reduced the frequency of acute attacks, the need for concomitant use of other medication such as steroids, and chronic airway obstruction (1). However, in spite of oral theophylline's good bioavailability, problems are associated with its use.

The rather short half-life of theophylline and its narrow therapeutic range [10–20 μg/ml in plasma (2, 3)] make it necessary to administer the drug relatively often, while the peak levels achieved shortly after administration are associated with the observed side effects (2). It has been suggested that a slow-release theophylline preparation that would eliminate round-the-clock dosing every 6 hr would be desirable in the treatment of asthma (3, 4).

There are at least two approaches to developing a slow-release form of any drug: by formulation (4, 5) and by a chemical modification of the drug, a "slow-release prodrug." Because of the well-known problems related to the first approach, the latter approach was chosen.

Theophylline is a fairly water-soluble, polar molecule. It has only one position available for reversible modification, the 7-position. The slow-release approach consisted simply in decreasing the water solubility and dissolution rate by increasing the lipophilicity and the intermolecular forces in the crystals, respectively, of theophylline by acylating the 7-position. Unlike other aliphatic and arylamides, 7-acetyltheophylline (6), the only carefully studied member of this series, hydrolyzes quickly; in this respect, it resembles acid chlorides in its reactivity.

The expected rapid hydrolyses of the 7-acyltheophyllines ensure the equally rapid release of theophylline from the solvated 7-acyltheophyllines; and by using lipophilic acyl portions in the theophylline derivatives, their solubility in water is decreased. Thus, the slow dissolution of the derivatives due to their hydrophobic nature, followed by the quick hydrolysis of the theophylline derivatives once they are in solution, results in a slowly released theophylline.

To confirm this hypothesis, some 7-acyltheophylline derivatives were prepared and their dissolution rates were determined. Based on the dissolution rates, active component content, toxicity, and stability, one derivative, 7,7′-succinyldithophylline, was tested further *in vivo*, and some interesting aspects of its chemistry were explored. 7,7′-Succinyldithophylline, which had a slow dissolution rate yet fast hydrolysis, indeed afforded an excellent slow-release form of theophylline.

EXPERIMENTAL[1]

Preparation of 7,7′-Succinyldithophylline (I)—*Method A*—To 2.03 g (17.0 mmoles) of thionyl chloride were added 2.77 g (38.0 mmoles) of dimethylformamide and 0.83 g (7.0 mmoles) of succinic acid, in that order, at room temperature while nitrogen was bubbled through the reaction mixture; the reaction warmed during the addition of the dimethylformamide. After 0.25 hr, 2.52 g (14.0 mmoles) of theophylline was

[1] All melting points are uncorrected. NMR spectra were run on a Varian T-60 spectrometer, using tetramethylsilane as an internal standard. IR spectra were obtained from a Beckman Acculab 4 spectrophotometer. The UV spectra were recorded on a Cary 14 spectrophotometer. Microanalyses were performed by Midwest Microlab, Indianapolis, Ind. Theophylline was obtained from Knoll, Inc.; unless otherwise specified, all other chemicals were obtained from Aldrich Chemical Co. and all solvents were analytical reagent grade and obtained from Mallinckrodt, Inc.

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added, and the resulting suspension was diluted with 100 ml of dichloromethane. Then 2.82 g (34 mmoles) of pyridine was added dropwise to the well-stirred suspension, causing the suspension to clear gradually and the resulting solution to take on a yellow color. All additions were done at room temperature, and nitrogen was bubbled through the reaction mixture the entire time.

The reaction solution was then protected from atmospheric moisture with a calcium chloride drying tube, and the solution was refluxed overnight with the heating bath temperature at 60–70°. The resulting suspension was filtered, and the residue was washed with dichloromethane to give 1.50 g (50% yield) of I as a white solid whose IR and UV spectra showed no theophylline impurity.

The IR spectrum of I was characterized by three bands at 3160 (m), 1734 (s) (NC=O), and 1540 (m) cm^{-1}. Theophylline itself showed a very broad, intense absorption between 3300 and 2200 cm^{-1} in which it was difficult to pick out a characteristic absorption; it did not show any absorption at 1734 cm^{-1} and showed the 1540-cm^{-1} absorption shifted to 1570 cm^{-1}. The UV spectrum of I in dichloromethane was characterized by a symmetrical band centered at 300 nm with an intensity of $\epsilon = 1.31 \times 10^4$, while theophylline was characterized by a symmetrical band centered at 270 nm with an intensity of 1.0×10^4.

Anal.—Calc. for $C_{18}H_{18}N_8O_6$: C, 48.87; H, 4.10; N, 25.33. Found: C, 49.19; H, 4.11; N, 25.02.

Method B—To 2.04 g (13 mmoles) of ice bath cooled and well-stirred succinyl chloride was added 2.33 g (32 mmoles) of dimethylformamide. This clear colorless solution was stirred for 10 min; then 4.68 g (26 mmoles) of theophylline was added, and the light-yellow suspension was diluted with 100 ml of dichloromethane. Finally, 2.21 g (27 mmoles) of pyridine was added dropwise to the well-stirred suspension. The suspension never did become homogeneous, but it did go from a reddish-orange to a grayish-green color during pyridine addition.

The suspension was gradually allowed to warm to room temperature and was stirred over the weekend at room temperature; the reaction was protected from atmospheric moisture at all times with a calcium chloride drying tube. Then the suspension was filtered, and the residue was washed with 50 ml of dichloromethane to give 4.88 g (85% yield) of I as a yellow solid. The IR spectrum was identical with that of I from Method A; UV (CH_2Cl_2) λ_{max} 300 nm ($\epsilon = 1.28 \times 10^4$). The yellow solid was suspended in refluxing dichloromethane for 2 hr. The hot suspension was filtered to give a yellow solid (4.77 g) which now had λ_{max} 300 nm ($\epsilon = 1.31 \times 10^4$) in dichloromethane.

Anal.—Calc. for $C_{18}H_{18}N_8O_6$: C, 48.87; H, 4.10; N, 25.33. Found: C, 48.44; H, 3.96; N, 25.31.

Method C—To 1.89 g (15.9 mmoles) of well-stirred thionyl chloride at room temperature were added 2.60 g (35.6 mmoles) of dimethylformamide, dropwise, and 0.80 g (8 mmoles) of succinic anhydride. The resulting suspension was stirred at room temperature for 1 hr, during which time it became homogeneous and light yellow in color. Then dichloromethane (50 ml) was added to the solution, followed by 2.70 g (15 mmoles) of theophylline, 50 ml of dichloromethane, and 2.80 g (35 mmoles) of pyridine.

Nitrogen was bubbled through the clear yellow-orange solution for 20 min at room temperature, and the solution was refluxed overnight in a reaction flask equipped with a calcium chloride drying tube. The next day, the resulting suspension was filtered while hot to give 1.70 g (51% yield) of I as a white solid. The IR spectrum was identical to that of I from Method A; UV (CH_2Cl_2) λ_{max} 300 nm ($\epsilon = 1.30 \times 10^4$).

Anal.—Calc. for $C_{18}H_{18}N_8O_6$: C, 48.87; H, 4.10; N, 25.33. Found: C, 48.79; H, 4.08; N, 25.63.

Method D—The same results as Method C were obtained when phosgene in benzene was substituted for thionyl chloride on a molar basis.

Anal.—Calc. for $C_{18}H_{18}N_8O_6$: C, 48.87; H, 4.10; N, 25.33. Found: C, 48.70; H, 3.91; N, 25.61.

In addition to I, the following 7,7′-acylditheophylline compounds were prepared according to Method B:

7,7′-Glutarylditheophylline (II)—mp 229–230°, 84% yield.

Anal.—Calc. for $C_{19}H_{20}N_8O_6$: C, 49.99; H, 4.42; N, 24.56. Found: C, 49.94; H, 4.71; N, 24.84.

7,7′-Adipylditheophylline (III)—mp 238–239°, 82% yield.

Anal.—Calc. for $C_{20}H_{22}N_8O_6$: C, 51.06; H, 4.71; N, 23.82. Found: C, 50.96; H, 4.95; N, 23.80.

7,7′-Terephthaloyldittheophylline (IV)—92% yield.

Anal.—Calc. for $C_{22}H_{18}N_8O_6$: C, 54.10; H, 3.30; N, 22.94. Found: C, 53.40; H, 3.64; N, 22.48.

Preparation of 7-Octanoyltheophylline (VI)—Theophylline (4.5 g, 0.025 mole) was added to 200 ml of anhydrous 1,2-dichloroethane containing 5 ml (0.062 mole) of pyridine. Octanoyl chloride (4.86 g, 0.03 mole) diluted with 50 ml of 1,2-dichloroethane was then added to the solution. The mixture was heated at reflux for 2 hr and cooled to 0°. The precipitate was filtered, and the filtrate was concentrated *in vacuo* to give a white crystalline residue. The crystals were recrystallized from heptane to give 6.2 g (mp 62–63°, 90% yield) of VI.

Anal.—Calc. for $C_{15}H_{22}N_4O_3$: C, 58.81; H, 7.28; N, 18.28. Found: C, 58.68; H, 7.24; N, 18.48.

In a similar manner were prepared the following 7-acyltheophylline derivatives:

7-Decanoyltheophylline (VII)—mp 71–72°, 87% yield.

Anal.—Calc. for $C_{17}H_{26}N_4O_3$: C, 61.06; H, 7.84; N, 16.75. Found: C, 60.99; H, 7.81; N, 16.87.

7-Palmitoyltheophylline (VIII)—mp 92–93°, 88% yield.

Anal.—Calc. for $C_{23}H_{38}N_4O_3$: C, 66.00; H, 9.15; N, 13.38. Found: C, 66.06; H, 9.24; N, 13.41.

7-Cinnamoyltheophylline (IX)—mp 234°, 91% yield.

Anal.—Calc. for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.76; H, 4.40; N, 18.16.

Preparation of Ethyl 7-Succinyltheophylline (X)—A dichloromethane (125 ml) suspension containing 4.0 g (0.022 mole) of theophylline and 2.25 g (0.022 mole) of triethylamine was added to an ice bath cooled dichloromethane (100 ml) solution of ethyl succinyl chloride (3.6 g, 0.022 mole). The solution was filtered immediately, and the filtrate was concentrated *in vacuo* at room temperature to give a hygroscopic residue, which was suspended in ether (500 ml). The suspension was filtered, and the residue was discarded.

The filtrate was concentrated *in vacuo* to give a white solid, which was triturated with heptane (200 ml). The suspension was filtered, and the residue was dried to give 1.19 g (mp 107–109°, 17% yield) of X; IR (KBr): 1720, 1695, and 1660 (s) (C=O) cm^{-1}; NMR ($CDCl_3$): δ 8.46 (s, 1, N=CHN), 4.06 (q, J = 7 Hz, 2, OCH_2CH_3), 3.73–3.57 (m, 2, CH_2C=ON), 3.57 and 3.37 (two s, 6, NCH_3), 2.83–2.60 (m, 2, CH_2C=OO), and 1.18 (t, J = 7 Hz, 3, CH_3CH_2O) ppm.

Anal.—Calc. for $C_{13}H_{16}N_4O_5$: C, 50.64; H, 5.23; N, 18.17. Found: C, 50.58; H, 5.27; N, 18.28.

Preparation of 7′-*p*-Toluenesulfonyltheophylline (XI)—A tetrahydrofuran (100 ml) suspension containing 1.54 g (0.015 mole) of triethylamine, 2.70 g (0.015 mole) of theophylline, and 2.70 g (0.014 mole) of *p*-toluenesulfonyl chloride was refluxed for 48 hr and then filtered while still warm to remove triethylamine hydrochloride. The filtrate was cooled, and the resulting precipitate was filtered. The residue (2.60 g) was suspended in 50 ml of benzene to remove unreacted theophylline.

The benzene filtrate was concentrated *in vacuo* to give 2.2 g (mp 202–204°, 46% yield) of XI as a white powder; TLC (silica gel, acetone): R_f 0.66; NMR ($CDCl_3$): δ 8.33 (s, 1, N=CHN), 7.78 (*AB* quartet, J = 9 Hz, $\Delta_{AB\nu}$ = 38 Hz, 4, aromatic H), 3.58 and 3.36 (two s, 6, NCH_3), and 2.45 (s, 3, aromatic CH_3) ppm.

Anal.—Calc. for $C_{14}H_{14}N_4O_4S$: C, 50.29; H, 4.22; N, 16.76. Found: C, 50.31; H, 4.15; N, 16.83.

Preparation of 7,7′-Carbonylditheophylline (V)—A chloroform (100 ml) suspension of theophylline (3.60 g, 0.02 mole) was treated with 10.3 g (12 ml, 12.5 mmoles) of 12.5% phosgene in benzene and then with a chloroform (20 ml) solution of pyridine (2.0 g, 0.025 mole). The solution that resulted was allowed to react at room temperature overnight in a tightly sealed reaction flask. The next day, an additional 4 ml of 12.5% phosgene in benzene was added. The suspension was allowed to stir at room temperature overnight, and then it was heated to reflux and quickly filtered while hot.

The residue was dried *in vacuo* over phosphorus pentoxide to give 1.72 g (45% yield) of V as a white powder, which was too unstable upon exposure to the atmosphere to give a satisfactory elemental analysis. The IR spectrum showed complete loss of NH absorption and the appearance of two new carbonyl bands at 1780 and 1750 cm^{-1}; UV (dioxane): λ_{max} 274 nm ($\epsilon = 2.91 \times 10^4$); UV ($CH_2Cl_2$): λ_{max} 270 nm ($\epsilon = 2.96 \times 10^4$). The $t_{1/2}$ dissolution in water was 4.5 min.

NMR Studies—The NMR spectrum were obtained by directly mixing the appropriate reagents on a 0.7–1.0-mmole scale in NMR spin tubes or in small (10 ml) erlenmeyer flasks from which NMR samples were taken when desired. Each reaction was run at least three times with no significant variation in the results. The reaction between dimethylformamide and thionyl chloride in deuterochloroform after 10 min resulted in the formation of very broad absorptions centered in the regions of 660–580 Hz (0.22, relative integration) and 510–460 Hz (0.78) for NCH and of 280–210 Hz (1.3) and 210–150 (4.7) for NCH_3.

The lower field absorption in each set was attributed to the ionic, $(CH_3)_2N$=$CHCl^+$ Cl^-, rather than the covalent, $(CH_3)_2NCHCl_2$, form

Table I—Dissolution of Acyltheophyllines

Compound	$t_{50\%}$	Preparation Method[a]
Theophylline	<10 sec	—
I	1 hr	B
I	22 hr[b]	C
I	22 hr[b]	D
II	45 min	B
IV	3 hr	B
VII	18 hr	B
VIII	60 hr	B
IX	75 hr	B

[a] See *Experimental*. [b] After proper formulation, a half-life of less than 4 hr could be obtained.

of the Vilsmeier reagent obtained. When the reagent was allowed to age, there was a decrease in the contribution of those absorptions attributed to the ionic form.

When an equivalent of succinic anhydride was added to the Vilsmeier reagent, an absorption at 184 Hz was observed. Furthermore, all succinic anhydride went into solution, whereas a dimethylformamide–deuterochloroform solution in the same ratio and volume did not dissolve that much succinic anhydride. Therefore, the absorption at 184 Hz was assigned to the $CH_2C{=}O$ absorption in the intermediate formed between the Vilsmeier reagent and succinic anhydride. As the reaction mixture of dimethylformamide, thionyl chloride, and succinic anhydride was allowed to age, another absorption appeared at 200 Hz. It was identified as due to succinyl chloride by spiking the sample with succinyl chloride. The absorption due to succinyl chloride amounted to 5% after 1 hr, 33% after 16 hr, and 63% after 41 hr.

When one-half equivalent of succinic acid was substituted for succinic anhydride in this reaction, the time course of succinyl chloride formation was much slower; after 16 hr, about 10% succinyl chloride was formed. The $CH_2C{=}O$ absorption due to the remaining succinyl moiety remained at about 185 Hz. But the CH_3N absorption due to dimethylformamide was no longer centered at about 184 Hz but was at 196 Hz instead; with time, it appeared to shift to 205 Hz.

The addition of two equivalents each of theophylline and pyridine to freshly prepared reaction mixtures of dimethylformamide, thionyl chloride, and succinic anhydride resulted in the formation of yellow-orange solutions with very small amounts of solid suspended in them. Almost all theophylline dissolved in each case, but the clear solutions obtained in preparative scale reactions were never obtained, probably because of the difficulty in maintaining anhydrous conditions. Analysis of the NMR spectra of those solutions immediately after they were formed showed, in addition to pyridine, the absorptions due to dimethylformamide (two sharp singlets at 179 and 173 Hz, CH_3N) and a theophylline-containing moiety (two sharp singlets at 218 and 202 Hz, CH_3N), as well as a sharp singlet at 188 Hz and a broad singlet at 152 Hz. The singlet at 188 Hz was identified as succinic anhydride by spiking the sample with succinic anhydride. With time, the broad singlet at 152 Hz became broader and shifted downfield; after 16 hr, it had shifted to 164 Hz. In each case, after about 24 hr, the precipitate obtained was analyzed and found to be 7,7′-succinylditheophylline (I).

Theophylline was not soluble to the same extent in the same mixture of dimethylformamide–deuterochloroform, nor did the addition of pyridine hydrochloride improve theophylline solubility in such a mixture. There was an unidentified reaction between theophylline and the Vilsmeier reagent.

Dissolution Studies—The dissolution studies were conducted directly in accordance with the guidelines set forth in the USP (7). The apparatus and materials employed were within USP requirements.

Table II—Stability of 7,7′-Succinylditheophylline

Preparation Method	Conditions	$\epsilon \times 10^4$ (Percent Decomposition)[a] 1 Week	2 Weeks	3 Weeks
A	60°[b]	1.28 (1.5)	1.25 (4.0)	—
B	60°[b]	0.95 (27)	0.71 (46)	—
C	60°[b]	1.28 (1.5)	1.21 (6.5)	1.16 (10)
	25°, 52% R.H.[c]	1.30 (0)	1.29 (0.7)	—
	40°[b]	1.30 (0)	1.24 (4.5)	1.20 (8)

[a] Samples analyzed in dichloromethane at 300 nm by UV; original transmittance was 1.30 for all samples. [b] Samples stored in sealed ampuls. [c] The relative humidity was maintained in a sealed desiccator with a standard salt solution, and the sample was kept exposed to that atmosphere in the desiccator.

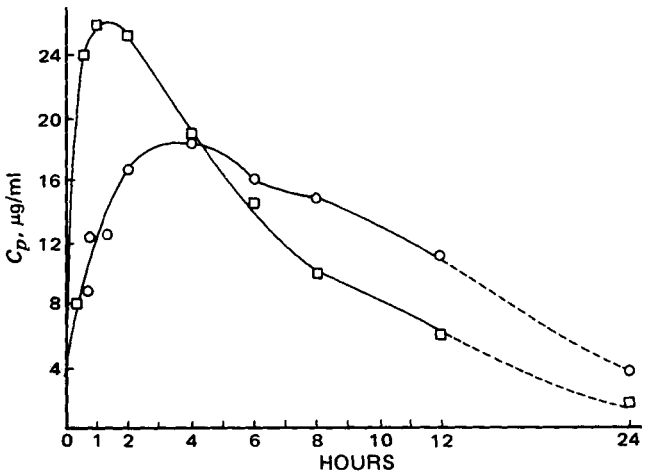


Figure 1—*Bioavailability of theophylline* (□) *and I* (○) *at doses of 30 mg/kg or equivalent.*

The dissolution rates of the theophylline compounds were obtained in 500 ml of distilled water containing 2 drops of polysorbate 80[2] in a standard dissolution pot at 25 ± 0.5° in a constant-temperature water bath. Samples of 100–200-mesh powder of each compound tested were transferred directly into the dissolution medium and stirred with a standard USP stainless steel paddle. The paddle was placed at the center of the 500 ml of dissolution medium and rotated at 100 rpm. After a constant reading was obtained, the solution was sonicated for 15 min to obtain the infinite reading.

All samples were run at least twice. The concentration of each sample in the dissolution medium never exceeded 5% of the solubility of theophylline.

The results of some representative dissolution studies are presented in Table I.

Bioavailability Studies—Beagle dogs of both sexes, 10–15 kg, were fasted for 12 hr prior to use. Theophylline (30 mg/kg) and 7,7′-succinylditheophylline (equivalent to 30 mg of theophylline/kg) were suspended in 5% methylcellulose and administered orally *via* a conventional gastric delivery tube. Each suspended drug solution was prepared immediately prior to administration. Blood (10 ml) was withdrawn from each dog immediately prior to drug administration. Then 10-ml blood samples were obtained at 15, 30, 60, 120, 240, 360, 480, and 720 min after drug administration.

The plasma was separated conventionally and stored in a freezer pending assay. Theophylline concentrations in plasma were determined by the spectrophotometric method of Shack and Waxler (8). Plasma (2 ml) was acidified with 1 *N* HCl to pH 5.5–6 and then was extracted with 20 ml of 5% 2-propanol in chloroform. The organic phase was reextracted with 3 ml of 1 *N* NaOH. The absorbance of the aqueous layer was determined with a 1-cm path length cell in a UV spectrophotometer.

The results of the bioavailability study are presented in Fig. 1. Compound I resulted in therapeutic plasma levels [10–20 μg/ml (2, 3)] essentially for 12 hr, while the same doses of theophylline resulted in toxic blood levels for about 4 hr and dropped below the therapeutic level after 7–8 hr.

Stability Studies—Stability studies were carried out by following the decrease in absorbance due to 7,7′-succinylditheophylline at 300 nm. Table II shows the results of some representative stability studies using five samples for each determination.

RESULTS AND DISCUSSION

Most of the theophylline derivatives (I–XI) were prepared by the reaction of an acid chloride with theophylline in the presence of an acid scavenger, usually pyridine. The compounds thus obtained have characteristic IR, UV, and NMR spectra (Table III). Their elemental analyses are also consistent with the structures indicated. For instance, theophylline itself has a very broad, intense NH absorption between 2400 and 2900 cm^{-1}. That absorption is obviously absent in the derivatives, which instead exhibit a strong carbonyl absorption at about 1730–1760 cm^{-1}.

The UV spectrum of theophylline exhibits a λ_{max} at 273 nm with an

[2] Tween 80.

Table III—Spectral Data for Theophylline Derivatives

Compound	NMR[a] NCHN	NMR[a] $CH_2C{=}ON$	NMR[a] CH_3N	IR[b], C=O
Theophylline	7.83	—	3.63, 3.47	1705, 1660
I	—	—	—	1735, 1705, 1660
II	8.43	3.50	3.60, 3.44	1735, 1716, 1700, 1660
III	—	—	—	1765, 1700, 1650
VI	8.37	3.43, t, J = 7 Hz	3.63, 3.47	—
VII	8.37	3.45, t, J = 7 Hz	3.61, 3.46	1755, 1715, 1670
VIII	8.37	3.41, t, J = 7 Hz	3.61, 3.46	1755, 1715, 1670
IX	8.57	—	3.63, 3.47	1710, 1700, 1665, 1650

[a] NMR spectra were run in deuterochloroform, and chemical shift values (δ) are given in parts per million. [b] IR spectra were run in potassium bromide, and absorption values are in reciprocal centimeters.

ϵ of 1×10^4, while the λ_{max} is shifted to about 298 nm for the monotheophylline derivatives and to slightly longer wavelengths for the ditheophylline derivatives. Comparison of the NMR spectrum of theophylline with the spectra of its derivatives shows that a characteristic change in the chemical shift of the 8-proton occurs [from δ 7.83 to 8.37 ppm in the octanoate VI, for example (Table III)] due to deshielding of the proton by the 7-acyl carbonyl in the derivatives.

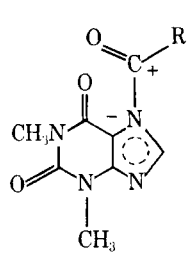
The chemical shift of the absorption due to the acyl α-methylene in the 7-acyltheophyllines is more difficult to explain. This methylene absorption is shifted 35–40 Hz downfield from the methylene absorptions in the corresponding acid chlorides. To obtain such a deshielded environment, it appears that it is necessary to invoke a mechanism whereby some charge separation occurs in the ground state of the CN bond (XII). An alternative possibility, in which acylation of the 6-carbonyl takes place to give the conjugated system XIII, would not generate a system with electron-withdrawing properties sufficient to cause the deshielding observed.

Furthermore, if there was enhanced charge separation in the ground state of the CN bond, one might expect elimination of theophylline to occur as readily as in an acid chloride (9), causing the formation of a ketene or, if two theophyllines are eliminated, a diketene-like intermediate, which would then readily polymerize. As a result of this mechanism, water-involved hydrolysis would not be the only route for I decomposition. The stability data presented in Table II suggest that decomposition indeed does not take place in the solid state primarily through a hydrolysis mechanism, since decomposition was not affected by humidity, but through a thermolysis mechanism. The stability data and the NMR spectra tend to support the theory that there is strong charge separation in the N-7 C=O bond of the 7-acyltheophyllines. The cinnamoyl derivative IX does not show this kind of thermal decomposition, obviously because of the lack of the possibility for ketene formation.

Of the monotheophylline derivatives, only NMR and IR data could be obtained for the hexanoyl derivative. The remaining monotheophylline derivatives could be recrystallized from alkanes. The ditheophylline derivatives were not conveniently soluble in any solvent tried. Fortunately, the ditheophylline derivatives could be obtained directly from the reaction mixtures in sufficient purity to establish their dissolution characteristics and other physical-chemical properties.

Table I shows the time course of dissolution of several representative derivatives. There was little effect of pH on the I dissolution rate at pH 2–7 under otherwise identical conditions. The hydrolysis of I under these conditions had a half-life in solution of about 10 sec[3]. The data for V (see *Experimental*) were obtained for the crude reaction product because it was too unstable to survive sieving and had to be stored in a desiccator.

The bioavailability of a slow-release drug depends, in this case, on how long the protected drug remains in the stomach. The time for gastric emptying is, of course, quite variable, so selecting an optimum dissolution half-time for the slow-release theophylline was somewhat arbitrary. However, based on previous work (4), a value of about 2 hr was considered optimal. Compound I contains a greater percentage of theophylline than any other derivative except V. No aryl residue is more than marginally less toxic than succinic acid, and the dissolution study suggests that I can be obtained in a form that has approximately the desired half-life for dissolution. Furthermore, since the dissolution of I was unaffected by pH in the 2–7 range, the I dissolution rate should be reasonably independent of the conditions found in the GI tract.



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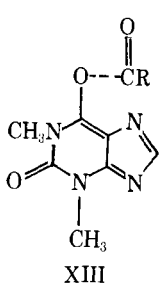


XIII

[3] V. J. Stella, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66044, personal communication.

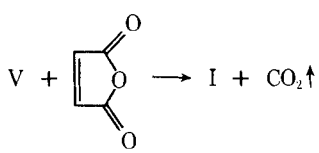
After the preliminary physical-chemical data were determined, *in vivo* data in beagle dogs were obtained for I to assess its utility more thoroughly. The results in Fig. 1 show that I with a half-time of dissolution of 2 hr indeed delivered theophylline in a slow, prolonged manner that resulted in a therapeutic blood theophylline level for a longer time than theophylline itself. A more detailed evaluation of the biopharmaceutics and pharmacokinetics will be presented elsewhere. Based on these data and considerations, I was selected as the best candidate for further evaluation. A successful attempt was made to obtain it in a pure stable form.

The preparation of I from succinyl chloride and theophylline in the presence of a suitable acid scavenger had a number of problems concerned with the physical nature of the product formed. Compound I, prepared in such a manner, was not particularly stable and its dissolution was erratic. These problems are normally overcome by recrystallization, but I was insoluble for all practical purposes in every common solvent. Thus, the crystal form of I that was produced during the synthesis was employed.

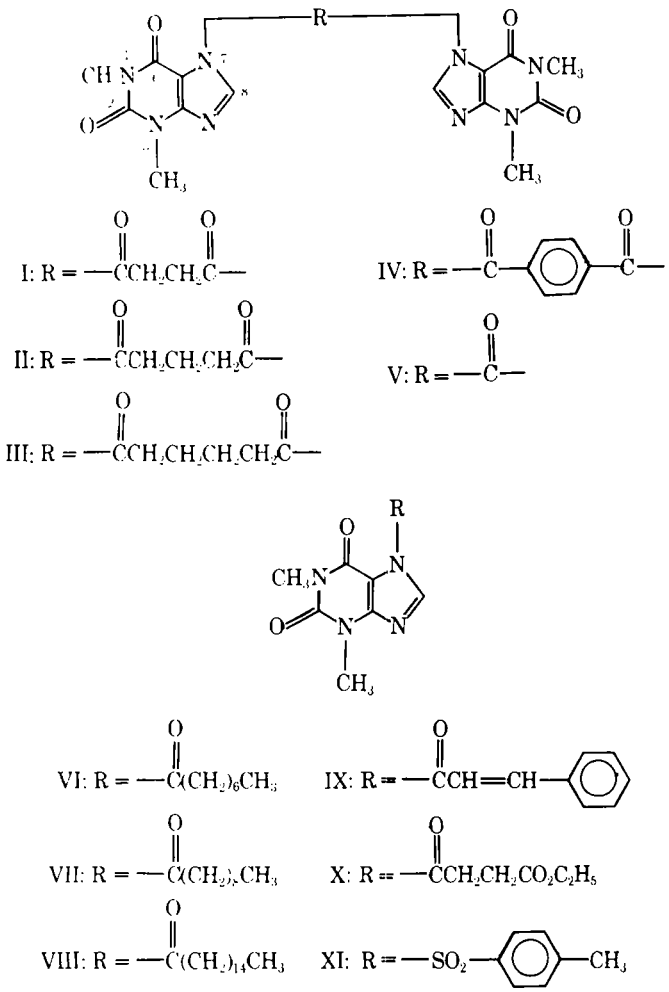
The question then became one of how to produce pure stable I with the desired dissolution rate directly from the reaction mixture. Usually, this synthesis is accomplished by adjusting the polarity of the solvent system and the concentration of the reagents so that the formation rate and subsequent precipitation of the product can be controlled. Inherent in this approach is that the reaction proceeds through a clear solution stage or that precipitation of the product is slow enough that it does not occlude unreacted starting materials. This effect could not be accomplished consistently in the preparation of I from theophylline and succinyl chloride.

Alternatively, an attempt was made to prepare I from different reagents. It was suggested that the highly reactive V could react under proper conditions with succinic anhydride, according to Scheme I, but the proper conditions for this exchange could not be found.

A recent patent describing the use of dimethylformamide as a catalyst for the preparation of succinyl chloride from succinic anhydride and phosgene suggested that a heat-labile intermediate was involved in the process (10). It was felt that, under suitable conditions, the intermediate could be caused to react with theophylline in the presence of an acid scavenger to give I. Thus, a mixture of stoichiometric amounts of dimethylformamide, thionyl chloride (Vilsmeier reagent) (11), and succinic anhydride contained only a trace of succinyl chloride initially (by NMR spectroscopy), but increasing amounts of succinyl chloride were observed after several days at room temperature. When the initial mixture of dimethylformamide, thionyl chlorde, and succinic anhydride was immediately allowed to react with theophylline in the presence of pyridine,



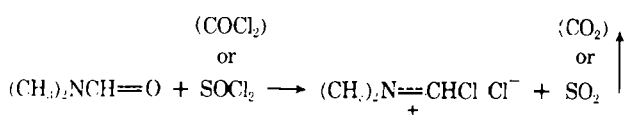
Scheme I



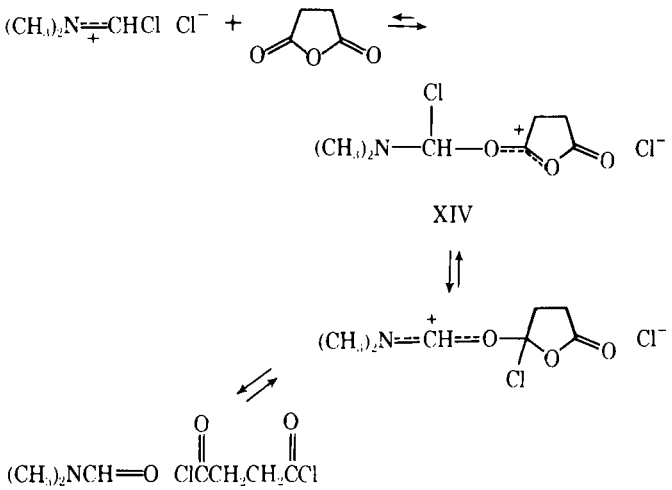
a clear solution was obtained within seconds. This solution did not contain any succinyl chloride (by NMR spectroscopy) and, upon heating, gave I as a precipitate.

The physical properties of I obtained in this manner were quite different from those of I obtained from the reaction of theophylline with succinyl chloride. In the former case, the crystals were visible to the naked eye. In the latter case, the product was an aggregate of amorphous particles. Obviously, these large crystals had a much different dissolution rate (22 hr) compared to the aggregate (1–2 hr). However, by a process of grinding, milling, and formulation, it was possible to convert the long dissolution rate crystals of I to a powder with the desired shorter dissolution rate. The process of converting the crystals of I into a product with a shorter dissolution rate did not perceptibly affect I stability. The stability data for I obtained by the various procedures described under *Experimental* were for forms of I with the same half-life of dissolution (Table II).

The NMR spectrum of the clear solution obtained in the initial stages of the reaction of the Vilsmeier reagent with succinic anhydride and theophylline in the presence of pyridine showed absorptions attributable to dimethylformamide (CH_3N), succinic anhydride ($CH_2C{=}O$), and pyridine and theophylline (CH_3N), as well as a broad absorption centered at 152 Hz. This later absorption and the solubilization of the theophylline in the reaction mixtures suggested that the theophylline absorptions (CH_3N) and the absorption at 152 Hz were due to the CH_3N and $O{=}CCH_2$ protons, respectively, of a soluble form of I or a precursor of I. One logical explanation for this behavior is that the soluble form may be the kinetic product, which would explain why it is necessary to heat



Scheme II



Scheme III

the reaction mixture for a considerable time to convert it to the thermodynamically more stable, insoluble form of I.

In the reaction between dimethylformamide–thionyl chloride, succinic anhydride, theophylline, and pyridine, no cooling was necessary; the reaction had to be refluxed to give good yields of I. The reaction between succinyl chloride, theophylline, and pyridine had to be cooled to obtain comparable yields of less stable I. If the latter reaction was cooled sufficiently (−40°), it occasionally proceeded through a clear solution stage which gradually, upon warming, gave I as a fine precipitate that was more stable than I obtained when the reaction was run at a higher temperature. It seemed reasonable that so much energy was released during the formation of the acylonium ion from succinyl chloride that the kinetic product was immediately converted to the more stable, insoluble form and in the process occluded some theophylline in its crystal lattice. The I thus produced was less stable and dissolved much quicker than I produced *via* the Vilsmeier reagent.

Although succinyl chloride was not detected in the reaction under the experimental conditions, it can be argued that succinyl chloride was produced as a slow step and then reacted quickly with theophylline in the presence of pyridine. This rationale did not fit, however, the observed fact that all theophylline went into solution immediately in a solvent system in which it was otherwise insoluble. Also, theophylline obviously was being converted into a soluble form more quickly than succinyl chloride was being generated; the appearance rate of succinyl chloride was just too slow to account for the rapid reaction observed.

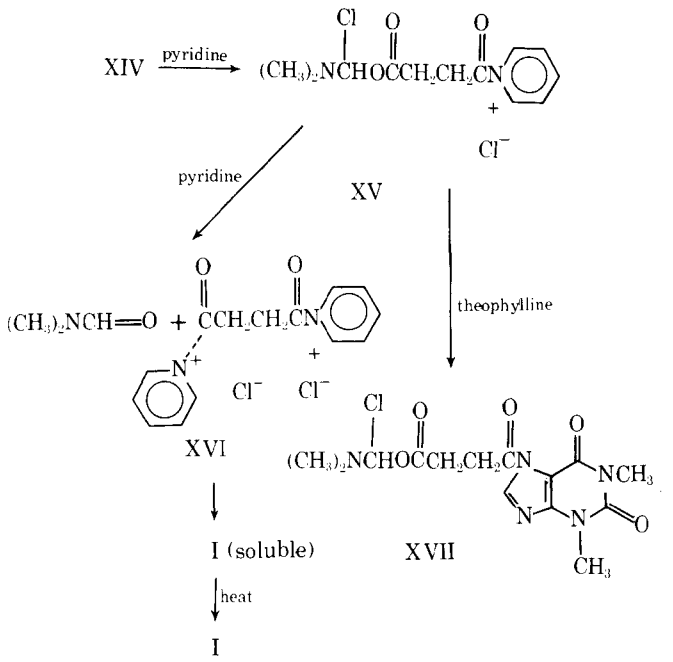
The reaction among dimethylformamide, thionyl chloride, and succinic acid, as followed by NMR, appeared to follow the same course.

The salient features of this unusual reaction with Vilsmeier reagent, succinic anhydride, theophylline, and pyridine are summarized as follows. Thionyl chloride reacts with dimethylformamide to form the Vilsmeier reagent in Scheme II. Then the Vilsmeier reagent reacts as a Lewis acid (12) with succinic anhydride in Scheme III to form the complex XIV, which can undergo a slow equilibrium reaction to give succinyl chloride. The reaction of XIV with pyridine to form the acylonium ion XV and the diacylonium ion XVI (Scheme IV) may be stepwise, with concomitant formation of XVII by trapping the monoacylonium ion with theophylline, or simultaneous, which results in the formation of I directly from XVI.

The reaction took place so quickly that it was not possible to observe any acyl monotheophylline intermediate by NMR spectroscopy. The simultaneous appearance of the NMR absorptions due to dimethylformamide and those attributed to the soluble form of I is compelling evidence that XVII does not represent the structure of the soluble form of I.

SUMMARY

A representative number of 7-acyltheophyllines and 7,7′-acylditheophyllines have been synthesized. One of them (I) has been identified as having properties of dissolution and stability that result in a prolonged controlled release of theophylline *in vivo*. Furthermore, a novel synthesis of I has been developed, which does not involve an acid chloride intermediate but which does appear to proceed through an acylonium ion generated under milder conditions than those obtained when the acylo-



Scheme IV

nium ion is generated from an acid chloride. The crystals of I obtained from this novel synthesis are more stable than those crystals of I obtained when the acid chloride is used in the synthesis of I. In addition, this novel synthesis results in I of consistent physical properties suitable for subsequent formulation.

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Assays and Statistical Analyses for Antibiotic Standards

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Received July 25, 1977, from the *National Center for Antibiotics Analysis, Food and Drug Administration, Washington, DC 20204.* Accepted for publication November 14, 1977.

Abstract □ Some microbiological assays and statistical analyses of test results used by the National Center for Antibiotics Analysis are described for the establishment of official antibiotic reference standards. Examples are given of both cylinder plate agar diffusion assays and turbidimetric assays. Formulas providing simple and quick analyses of data are shown for calculating potency, determining limits for the potency, and performing validity tests on the results.

Keyphrases □ Microbiological assays—for official antibiotic reference standards, formulas for quick, simple analysis of data □ Antibiotic reference standards, official—microbiological assays, formulas for simple, quick analysis of data

The Food and Drug Administration's National Center for Antibiotics Analysis (NCAA) establishes and maintains official reference standards for all antibiotics subject to certification. When a standard is required for a new antibiotic or when an existing standard must be replaced, NCAA performs a series of assays on representative samples of the proposed batch. The collaboration of other laboratories in assaying samples is requested; the findings are evaluated and certain statistical analyses are performed by NCAA.

In addition, NCAA is frequently called upon to participate and collaborate with other laboratories to establish official standards, such as international standards established by the National Institute for Medical Research in London, England, through the Expert Committee on Biological Standardization of the World Health Organization. Standards are also assayed at the request of the USP and NF.

Kirshbaum *et al.* (1) simplified the bioassay designs described by Bliss (2) and statistical procedures given in USP XV (3). For microbiological assays of antibiotics, they adapted a series of equations to determine potency, error variance, validity, and confidence limits of the assays. This adaptation was published in the USP XVI (4) and is still in use in NCAA laboratories. The calculations are based on a polynomial equation for fitting a line to a parabola. Coefficients and constants are derived from the table of orthogonal polynomials.

METHODS

When evaluating a proposed FDA standard, each participant is supplied with a quantity of the proposed standard and a reference standard and is requested to follow a specific design and to furnish NCAA with the necessary raw data. This design is applied to three-dose assays, where there is a linear response to the log of the dose, where there are parallel

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