

Alkaline, Enzymatic Hydrolysis and Physicochemical Properties of 7-Theophyllineacetic Acid Derivatives

BOIKA TSVETKOVA¹, JASMINA TENCHEVA^{1*} AND PLAMEN PEIKOV²

¹Department of Chemistry, Faculty of Pharmacy, Medical University-Sofia, Bulgaria

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University-Sofia, Bulgaria

(Received: December 10, 2002; Accepted: June 23, 2003)

ABSTRACT

In the present study, a number of esters which derived from 7-theophyllineacetic acid were prepared. The stability of 7-theophyllinylacetyloxyglycols was evaluated in sodium hydroxide solution (pH 13.0) at 60°C. The degradation reaction was monitored by using a HPLC assay with ultraviolet detection. All of esters were hydrolyzed to yield the correspondence quantitative amount of the hydrolytic product. The rate constants of the hydrolytic decomposition of the described esters were estimated. The chemical mediated conversion displayed first-order kinetics, and half-lives of hydrolysis were determined, too. Also, the ability of the esters to release 7-theophyllineacetic acid through diluted human plasma at 37°C was studied. Compounds were found to possess a high stability at these conditions. It was noticed that no spontaneous hydrolysis of ester derivatives was noticed within 24 hr as revealed by HPLC analysis. The aqueous solubility and lipophilicity (expressed as logP) of 7-theophyllineacetic acid and its esters were investigated by means of UV spectrophotometry. It was found that all of the esters were more lipophilic than its parent molecule. However, their solubility in aqueous media was decreased, compared by the parent drug.

Key words: 7-theophyllinylacetyloxyglycols, alkaline hydrolysis kinetics, enzymatic stability, partition coefficient, solubility, HPLC, UV spectrophotometry

INTRODUCTION

Acefylline piperazine, salt of 7-theophyllineacetic acid, has been clinically used as antiasthmatic agent, cardiac stimulant and diuretic⁽¹⁾. Several studies were reported⁽²⁾, however, indicated that the drug is poorly absorbed (apparent bioavailability of 2%) after intravenous administration. A proper strategy to improve the absorption of the drugs as well as to reduce toxicity and side effects would be to manipulate their physicochemical characteristics by chemical modification. In the course of our continuous interest to enhance the therapeutic efficacy of 7-theophyllineacetic acid, we have recently synthesized four novel esters of the drug with several glycols⁽³⁾. Recently, we investigated their *in vitro* stability at different pH values closed to the physiological one and temperature of 37°C⁽⁴⁾. The results indicated that the proposed esters are quite resistant to chemical degradation. In order to clarify the possibilities of esters to be hydrolysed, *in vitro* accelerated stability studies must be carried out. In this paper, the *in vitro* alkaline and enzymatic stability evaluations of derivatives were performed. Aqueous solubility and lipophilicity of 7-theophyllineacetic acid and its esters were also established.

MATERIALS AND METHODS

I. Chemistry

* Author for correspondence. Tel: +359-2-9883142;
Fax: +359-2-9879874; E-mail: zhtencheva@pharmfac.acad.bg

TLC was performed on DC-Alufolien Kieselgel 60 F254 (Merck) (0.20 mm) sheets with solvents: n-butanol-chloroform-acetone-25% ammonia (4:3:3:1 v/v/v/v). Detected at UV 254 nm. The given yields are those of TLC homogeneous product.

(I) General method for preparation of 7-theophyllinylacetyloxyglycols

Corresponding glycol was mixed with triethylamine (0.01 mol, 1.01 g) in 50 mL of anhydrous benzene. 7-Theophyllineacetyl chloride (0.01 mol, 2.57 g) was dissolved in 10 mL anhydrous benzene at room temperature. The reaction mixture was stirred under reflux, after 4 hr the solvent was evaporated and 50 mL chloroform was added to the residue. After filtration the filtrate was washed with 50 mL water twice and was dried over anhydrous sodium sulfate. Evaporation of the solvent, the crude product was crystallized from methanol.

1. 1-(7-Acetyloxy-1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purine)-2-hydroxyethane (1b)

From 7-theophyllineacetyl chloride and 0.02 mol (0.68 g) 1,2-ethanediol. Yield 1.75 g (42%); mp 212-214°C (212-213.5°C)⁽³⁾.

2. 1-(7-Acetyloxy-1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purine)-3-hydroxypropane (1c)

From 7-theophyllineacetyl chloride and 0.02 mol (1.52 g) 1,3-propanediol. Yield 2.28 g (50%); mp 190-192°C (190-191°C)⁽³⁾.

3. *1-(7-Acetyloxy-1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purine)-2-(2-methoxyethoxy)ethane (1d)*

From 7-theophyllineacetyl chloride and 0.01 mol (1.20 g) 2(2-methoxyethoxy)ethanol. Yield 2.40 g (45%); mp 235-237°C (235-237°C)⁽³⁾.

4. *1-(7-Acetyloxy-1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxo-7H-purine)-2-[2-(2-methoxyethoxy)ethoxy]ethane (1e)*

From 7-theophyllineacetyl chloride and 0.01 mol (1.64 g) 2[2(2-methoxyethoxy)ethoxy]ethanol. Yield 3.13 g (52%); mp 195-197°C (195-197°C)⁽³⁾.

II. Reagents

The starting materials for synthesis of compounds were commercially available research-grade chemicals, Fluka Chemie AG (Switzerland). HPLC grade methanol was used to prepare the mobile phase. Buffer reagents and all other chemicals were of reagent grade. Buffer solutions were prepared as follows: pH 13.0 (0.1M NaOH) and pH 7.4 (0.1M Na₂HPO₄ and 0.1M NaH₂PO₄). Human plasma LOT M99I13070 was obtained from Local blood center.

III. Apparatus

Melting points were determined with a Büchi 535 apparatus and were uncorrected. UV measurements were performed on a Shimadzu UV-VIS 1202 spectrophotometer. Chromatography was carried out isocratically, on modular HPLC system LC-10A Shimadzu (Japan) arranged of a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector with 20 µL loop, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. The analysis was controlled and the data were acquired with CLASS LC-10A software. A chromatographic column LiChrosorb RP-18, 125 × 4 mm, 5 µm (Merck, Germany), equipped with additional guard column (20 × 4 mm) to protect the analytical column during enzymatic stability studies was used.

IV. Determination of Aqueous Solubility

The aqueous solubility of 7-theophyllineacetic acid (1a) and its esters (1b-e) were determined at 25°C by adding excess amount of the respective compound to water in screw-capped test tube. The suspension was rotated on thermostatically controlled magnetic bar for 24 hr. It was ensured that the saturation equilibrium was established. Upon filtration an aliquot of the filtrate was diluted with an appropriate amount of water and the sample was analysed

by UV spectrophotometry at 274 nm. The concentration of the compounds in their saturated solutions was calculated by comparing the absorbance of the filtrate with a calibration curve.

Owing to the low water solubility of solutes, the solutions used to build up the calibration curve were prepared by dissolving a known amount of compound in methanol. These solutions were subsequently diluted with water.

Our previous studies indicated that the examined esters remained stable during the course of experiment⁽⁴⁾.

V. Determination of Lipophilicity

Lipophilicity is one of the most important physico-chemical properties affecting a drug biological activity. It is commonly expressed as the logarithm of the partition coefficient (log P) between organic and aqueous phase. In this investigation, the log P values, were determined by partitioning of a 7-theophyllineacetic acid (1a) and its derivatives (1b-e) between 1-octanol and phosphate buffer (pH 7.4; 0.1 M) using the classical shake-flask method⁽⁵⁾. It is known that 1-octanol-water system imitates in the highest degree partitioning of the drugs in the real biological systems⁽⁶⁾.

The two phases had been previously saturated with each other and then separated. A known concentration of derivative in phosphate buffer solution was shaken for 4hr with suitable volume of 1-octanol at room temperature. After achieving equilibrium, the mixtures were allowed to stand for 2 hr, during which time the phases were separated.

The absorbance of the compounds in the buffer phase was determined spectrophotometrically at 274 nm against a saturated buffer blank. All determinations were run in triplicate and the average value was recorded. The sample concentration was determined by comparison to a calibration curve. The partition coefficient was calculated from the distribution results by employing the following equation:

$$P = \frac{C_1 - C_2}{C_2}$$

where C₁ and C₂ are, the solute concentration in buffer phase before and after partitioning respectively.

VI. Stability Studies

(I) Hydrolysis in buffer

In the kinetic runs, the reactions were initiated by adding 5 mL stock solution of the compounds in methanol to 50 mL of preheated sodium hydroxide buffer solution (pH 13.0; 0.1 M), the final concentrations being about 1.3 × 10⁻⁴M. The reaction flasks were maintained in water bath at 60 ± 0.2°C. Samples were taken at suitable time

intervals during 6 hr of incubation. The progress of hydrolysis was monitored by means of HPLC, according to previously described procedure⁽⁴⁾. 20 μ L of each sample examined was analysed for remaining ester derivative. Quantitation of each compound was achieved with reference to a suitably prepared calibration curve, using peak areas. First order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual ester derivatives against time.

(II) *In vitro* hydrolysis of human plasma

The stability of the esters was studied in human plasma diluted to 80% with phosphate buffer (pH 7.4, 0.1M). The reaction was initiated by adding a 60 μ L stock solution of the ester in methanol-water to 5mL of preheated plasma solution, the final concentration of the compound being about 0.5×10^{-4} M. The mixtures were kept in water bath at 37°C and at appropriate intervals samples of 250 μ L were withdrawn and added to 500 μ L of a 2% solution of ZnSO₄ in methanol:water (1:1 v/v) in order to deproteinize the samples. After mixing and centrifugation at 1.3×10^4 rpm for 3 min, 20 μ L of the clear supernatant was analysed by HPLC for remaining ester derivative under the same chromatographic conditions⁽⁴⁾, except the mobile phase. In this case it consists of methanol and water (60:40 v/v).

Quantitation of each ester was achieved with a reference to a suitable prepared calibration curve. All determinations were run in triplicate. The average analytical recovery of esters from plasma was found to be 98 %.

RESULTS AND DISCUSSION

I. Synthesis

7-Theophyllineacetic acid and 7-theophyllineacetyl chloride were synthesized as literature⁽⁷⁾. The ester derivatives of 7-theophyllineacetic acid were obtained according to classical synthetic approach by interaction of the 7-theophyllineacetyl chloride with 1,2-ethanediol, 1,3-propanediol, 2(2-methoxyethoxy)ethanol and 2[2(2-methoxyethoxy)-ethoxy]ethanol in anhydrous benzene in the presence of triethylamine. In the previous study these compounds were prepared by esterification of the acid with corresponding glycols in the presence of *N,N*-dicyclohexylcarbodiimide and 4-(*N,N*-dimethylamino)pyridine as catalyst with yield of about 60% for 5-6 hr⁽³⁾. The progress of reaction was monitored by TLC (until exhaustion of the acid).

II. Alkaline Hydrolysis Studies

Our previous investigations indicated that the proposed esters appeared stable under conditions simulating those prevailing *in vivo*. In order to show that the esters can be converted to the parent drug *in vitro*, kinetic studies at more drastic conditions were performed. All of the derivatives

described underwent hydrolysis to completion in sodium hydroxide solution (pH 13.0; 0.1M) at 60°C. The success of hydrolysis in basic medium could be attributed to the immediate conversion of the acid formed to the carboxylic salt. The disappearance of ester was accompanied by the progressive appearance of free 7-theophyllineacetic acid as revealed by HPLC analysis. Fig. 1 illustrates the degradation of the compounds 1b-e at pH 13.0 and 60°C.

At constant pH value and temperature the formation of the acid was found to be linear function of the initial ester concentration indicating first-order degradation kinetics. First-order rate constants *K* for the hydrolysis were calculated from the slopes of semilogarithmic plots of percent ester remaining versus time. Typical first-order plot for the degradation of 1b is shown in Fig. 2. The corresponding half-lives were obtained using the formula: $t_{1/2} = \frac{\ln 2}{K}$. The rate constants for conversion of the esters to the parent compound as well as the half-lives of hydrolysis were found to be nearly identical as it seen from the Table 1.

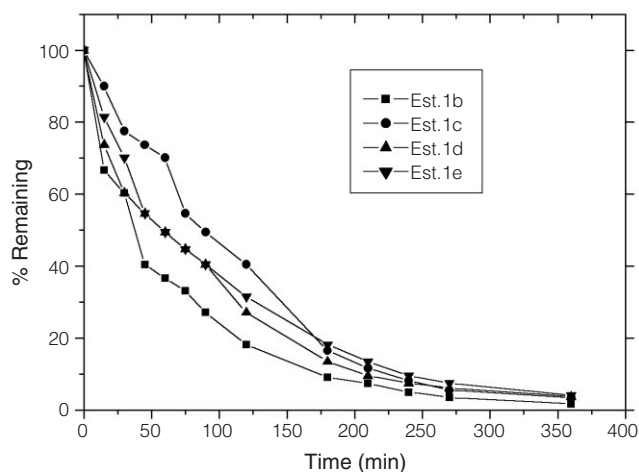


Figure 1. Time courses of disappearance of 7-Theophyllinylacetylglycols (1b-e) in sodium hydroxide buffer solution at 60°C.

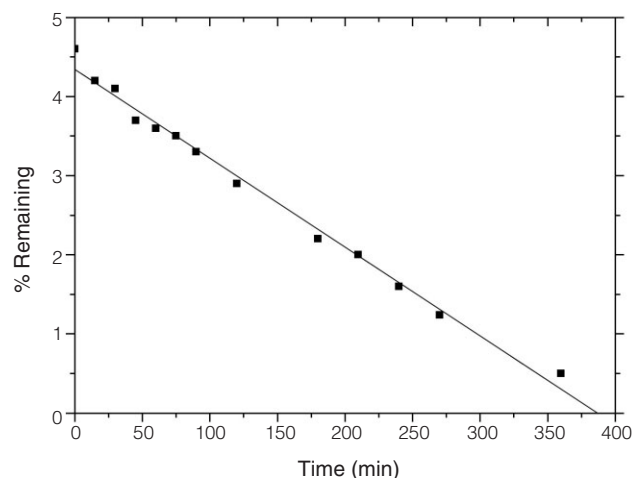
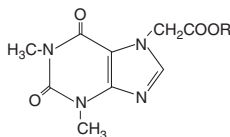


Figure 2. First-order plot for the degradation of compound 1b at pH 13.0 at 60°C

Table 1. Physicochemical and kinetic data of 7-theophyllineacetic acid (1a) and its esters (1b-e) in sodium hydroxide solution.

Compound	R	Solubility (mg/mL)	Log P	pH 13.0	
				K. 10 ⁻³	t _{1/2} (min)
1 a	H	1.47	-0.92		
1 b	CH ₂ CH ₂ OH	0.58	0.60	11.24	61.67
1 c	CH ₂ CH ₂ CH ₂ OH	0.42	0.73	10.38	66.78
1 d	CH ₂ CH ₂ OCH ₂ CH ₂ OCH ₃	0.33	0.88	9.57	77.36
1 e	CH ₂ CH ₂ OCH ₂ CH ₂ OCH ₂ CH ₂ OCH ₃	0.24	0.93	8.96	72.43



III. *In vitro* Enzymatic Stability Studies

In the present investigations, human plasma was used as a representative source of esterase activity present in the body. Two shortcomings of using plasma were recognized. Esterase activity in other tissues will differ in both magnitude and specificity from that in plasma⁽⁸⁾. Also, the total metabolic pathways available in the body will not be restricted to those present in plasma. In spite of these shortcomings, human plasma represents a convenient and common biological fluid for preliminary assessing the possible fate of the 7-theophyllinylacetyloxyglycols in a biological medium⁽⁹⁾.

All of the esters proposed proved to be quite stable in the presence of human plasma. No detectable change in the initial ester concentration was observed for 24 hr of incubation as evidenced from HPLC analysis.

A possible explanation of absence of hydrolysis of esters may be that they are not substrates for the enzyme. Human plasma exhibited an inhibitory effect on the hydrolysis of esters probably due to binding of the compounds to proteins.

IV. Physicochemical Properties

The aqueous solubility and partition coefficients of a 7-theophyllineacetic acid (1a) and the esters (1b-e) are listed in Table 1. It is obvious that the esterification of the carboxyl group with different glycols provided a possibility to modify the physicochemical characteristics of the parent compound.

Generally, the observed decrease in aqueous solubility of the investigated esters was accompanied by an increase in lipophilicity relative to the parent drug as illustrated by data in Table 1.

All of the esters proposed were more lipophilic than the parent compound, at the same time possessed less aqueous solubility as compared to 7-theophyllineacetic acid. The ester derivatives proved to be stable in 80% human plasma at 37°C during the time of experiment, and hydrolysed readily to the parent drug in sodium hydroxide solution (pH 13.0; 0.1M) at 60°C.

REFERENCES

1. Index Nominum 2000. 17th ed. p. 5. Swiss Pharm. Soc. ed. MedPharm Scientific Publishers, Stuttgart.
2. Sved, S., McGilveray, I. J. and Beaudoin, N. 1981. The assay and absorption kinetics of oral theophylline-7-acetic acid in the human. *Biopharm. Drug Dispos.* 2: 177-184.
3. Zlatkov, A., Peikov, P., Danchev, N., Ivanov, D. and Tsvetkova, B. 1998. Synthesis, toxicological, pharmacological assessment, and *in vitro* bronchodilating activity of some 7-theophyllinylacetyloxyglycols. *Arch. Pharm.* 331: 313-318.
4. Tsvetkova, B., Tencheva, J. and Peikov, P. 2001. *Acta Pharm.* 51: 317-321.
5. Leo, A., Hansch, C. and Elkins, D. 1971. Partition coefficients and their uses. *Chem. Rev.* 71: 525-616.
6. Nelson, R., Hansch, C. and Mattew, M. 1975. Selection of a reference partitioning system for drug design work. *J. Pharm. Sci.* 64: 599-606.
7. Maslankiewicz, A., Rohloff, B. and Windisch, B. 1975. Synthesis of theophylline-7-acetic acid. *Roczniki Chemii*, 49: 1935-1938.
8. Casida, J. E., Engel, J. L. and Nishizawa, Y. 1966. 3',5'-Diesters of 5-fluoro-2'-deoxyuridine and thymidine: hydrolysis by esterases in human, mouse and insect tissue. *Biochem. Pharmacol.* 15: 627-644.
9. Nielson, N. M. and Bundgaard, H. 1986. Prodrugs as drug delivery systems. 42. 2-Hydroxymethylbenz-amides as potential prodrug forms for amines. *Int. J. Pharm.* 29: 9-18.