# THE SYNTHESIS AND THE PHARMACOLOGICAL PROPERTIES OF ENANTIOMERIC DERIVATIVES OF 7-(2,3-DIHYDROXYPROPYL)THEOPHYLLINE

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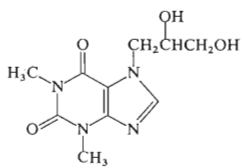
7-(*S*)-(2,3-Dihydroxypropyl)theophylline ((*S*)-*I*) and its enantiomer (*R*)-*I* were prepared by heating of the sodium salt of theophylline with 1-O-toluenesulfonyl-2,3-O-isopropylidene-D-glycerol or its L-enantiomer and subsequent acid hydrolysis. The two enantiomers *I* do not differ either by the inhibition of 3',5'-cAMP-phosphodiesterase, vasodilatatory activity on isolated guinea-pig aorta, or the effect on blood circulation of dogs *in vivo*.

7-(*RS*)-(2,3-Dihydroxypropyl)theophylline (*I*) (Diprophylline, Diphylline, Luphylline, Dilor, Neutrophylline, Neophylline, Glyphylline) has been described in literature and studied as a potential analogue of theophylline with a bronchodilatatory effect[1,2]. Although its activity is lower in comparison with theophylline and the average half-time in the organism considerably shorter than in theophylline[3], lower side-effects were observed during clinical application than in theophylline[4]. Compound *I* can exist in two optically active forms. In systematic studies with hydroxylated alkyl derivatives of heterocyclic bases it was observed that some of these compounds display biological activity, characteristic of individual enantiomers[5,6]. A typical example is 9-(2,3-dihydroxypropyl)adenine the (*S*)-form of which has a high unspecific antiviral effect[5,7]. Since the preparation of enantiomeric 2,3-dihydroxypropyl derivatives of heterocyclic bases has been sufficiently methodically elaborated[8,9] we decided to prepare both optically active forms of diprophylline (*I*) and to compare some of its biological parameters *in vivo* and *in vitro* with the racemic derivative as comparative material.

For the preparation of (*S*)- and (*R*)-diprophylline (*I*) the sodium salt of theophylline was condensed with 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-D- or -L-glycerol, prepared from D-mannitol by reaction sequence described in literature[8-10]. This reaction gives rise to 2,3-O-isopropylidene derivative of compound *I* which on boiling with acetic acid affords the corresponding optically pure product. Condensation takes place in the position 7 of theophylline exclusively, similarly as alkylations[11] or the reaction with 1,2-cyclic carbonate of glycerol[12]. The two enantiomers *I*

have identical UV spectra, characteristic of the derivatives of coffein. Their $^1$H-NMR spectra with all the expected characteristic signals are identical with the spectrum of the racemate of compound *I* (see[12]), and the chromatographic properties and the absolute molar rotation values are also practically identical. The racemic derivative *I* was obtained by a similar reaction using 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene--*sn*-glycerol[8], and according to the given criteria it was identical with an authentic sample[12]. For the comparison of biological properties both enantiomers and the racemate of compound *I* were prepared by the same method, then worked up in the same manner, and finally recrystallized from ethanol three times.



*I*

As a 7-alkyl derivative of theophylline, compound *I* is an analogue of coffein and thus also a potential inhibitor of 3',5'-cAMP-phosphodiesterase (EC 3.1.4c), *i.e.* an enzyme which by its effect on the metabolism of adenosine 3',5'-cyclic phosphate plays an important role in regulation mechanisms. Therefore we investigated the effect of all three stereoisomeric forms of compound *I* on the cleavage of adenosine 3',5'-cyclic phosphate with phosphodiesterase of dog heart under optimum conditions of cleavage. Under the given conditions ($K_m = 3{\cdot}25 \pm 0{\cdot}10 \,.\, 10^{-3}$M) the inhibitory effects of both enantiomers of compound *I* do not differ ($K_i = 1{\cdot}07 \,.\, 10^{-2}$M for the (*S*)-form, $1{\cdot}04 \,.\, 10^{-2}$M for the (*R*)-form) from one another or from the racemate ($K_i = 1{\cdot}04 \,.\, 10^{-2}$M) either; in all instances the inhibition has a non-competitive nature and it is comparable (by its order of magnitude) with the inhibitory effect of coffeine ($K_i = 8{\cdot}7 \,.\, 10^{-3}$M), which is also non-competitive. Hence, it may be assumed that during the formation of the complex of the inhibitor with the enzyme) only the heterocyclic base participates in the interaction, whereas the chiral part of the molecule *I* does not, so that the inhibition corresponds in character to achiral coffein.

For the estimation of the vasodilatatory effect of the forms of compound *I* *in vitro* isolated guinea-pig aorta tonised with noradrenaline in Krebs's solution was used. The data in Table I show the comparison of the concentrations eliciting half of the relaxation in individual forms and their comparison with the values for nylidrine and theophylline. The (*R*)-form and the racemate of compound *I* are equally active, while the (*S*)-form has hardly one half of their activity. Their

activity is roughly equal as in theophylline, but by two orders of magnitude lower than in nylidrine. In *in vivo* experiments on dogs anesthetized with pentobarbital the substances were administered intravenously (20 mg/kg) and their ECG, blood pressure in the *arteria femoralis*, and the blood flow in the *arteria femoralis* were followed. The results given in Table II show that all three forms of compound *I* mildly increase the flow-rate in *a. femoralis*, while the duration of the effect does not exceed 10 min. The substances do not affect either the pulse frequency of the systolic blood pressure, and mildly decrease the diastolic blood pressure. Although in the last mentioned parameter the effect of the (*R*)- and the (*RS*)-form is higher than in the (*S*)-form, no statistically significant difference in the biological effect was found between individual forms, similarly as with other parameters.

Thus it may be concluded that none of the newly prepared optically active forms of 7-(2,3-dihydroxypropyl)theophylline (*I*) differs statistically significantly in the mentioned tests from the racemic form.

## EXPERIMENTAL

The melting points were determined on a Kofler block and they are uncorrected. The solutions were evaporated at 40°C/2 kPa and the samples were dried at 0·1 Torr over phosphorus pentoxide.

Paper chromatography was carried out on paper Whatman No 3 MM with 2-propanol–conc. ammonia–water (7 : 1 : 2) ($S_1$), thin-layer chromatography on Silufol UV 234 plates (Kavalier, Czechoslovakia) in the systems chloroform–ethanol (9 : 1) ($S_2$) and chloroform–ethanol (4 : 1) ($S_3$). Detection was carried out under UV light (Chromatolite). The UV spectra were measured on a Unicam SP 8000 spectrophotometer in aqueous solutions, the $^1$H-NMR spectra on a Varian 100 instrument in deuteriochloroform (with tetramethylsiloxane as internal reference). The chemical shift values are given in ppm and the coupling constants in Hz.

TABLE I
Vasodilatatory Effect *in vitro*

| Compound | Number of determinations | $ED_{50}$, M . $10^8$ |
|---|---|---|
| Nylidrine | 2 | 0·1 |
| Theophylline | 8 | 55·0 |
| (*S*)-*I* | 4 | 67·0 |
| (*R*)-*I* | 6 | 28·0 |
| (*RS*)-*I* | 10 | 28·0 |

7-(*S*)-(2,3-Dihydroxypropyl)theophylline ((*S*)-*I*)

Sodium hydride (1·3 g; 60 mmol) was added to a suspension of 9·0 g (50 mmol) of theophylline in 100 ml of dimethylformamide and the mixture was stirred until the solution became clear (15 min). After addition of 17·4 g (60 mmol) of 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene--D-glycerol[8] the mixture was stirred at 100°C and under elimination of humidity for 6 h. It was evaporated at 40°C/13 Pa. The residue was extracted with chloroform (200 ml), filtered off under suction, and the precipitate washed with 100 ml of chloroform. The filtrate was evaporated in a vacuum and refluxed with 200 ml of 80% acetic acid for 90 min. After evaporation the resi-

TABLE II
Parameters of Blood Circulation

| Compound | Before application | After application, %[a] | | | |
|---|---|---|---|---|---|
| | | 0 min | 3 min | 5 min | 10 min |
| | | Pulse frequency (pulse/min) | | | |
| (*S*)-*I* | 178 ± 14 | 102 ± 1 | 103 ± 1 | 102 ± 2 | 102 ± 1 |
| (*R*)-*I* | 177 ± 17 | 102 ± 2 | 106 ± 2 | 106 ± 2 | 101 ± 2 |
| (*RS*)-*I* | 170 ± 17 | 101 ± 3 | 103 ± 2 | 101 ± 2 | 101 ± 2 |
| | | Systolic blood pressure, Torr | | | |
| (*S*)-*I* | 194 ± 11 | 102 ± 2 | 103 ± 1 | 102 ± 2 | 99 ± 2 |
| (*R*)-*I* | 201 ± 15 | 102 ± 2 | 101 ± 2 | 101 ± 3 | 95 ± 3 |
| (*RS*)-*I* | 192 ± 13 | 106 ± 2 | 104 ± 2 | 102 ± 2 | 100 ± 2 |
| | | Diastolic blood pressure, Torr | | | |
| (*S*)-*I* | 112 ± 8 | 101 ± 3 | 100 ± 2 | 98 ± 3 | 96 ± 2 |
| (*R*)-*I* | 112 ± 9 | 96 ± 3 | 90 ± 6 | 95 ± 5 | 93 ± 4 |
| (*RS*)-*I* | 107 ± 8 | 96 ± 5 | 98 ± 3 | 104 ± 3 | 98 ± 4 |
| | | Instantaneous blood flow in *a. femoralis*, ml/min | | | |
| (*S*)-*I* | 79 ± 17 | 110 ± 4 | 111 ± 9 | 115 ± 10 | 102 ± 8 |
| (*R*)-*I* | 85 ± 14 | 116 ± 6 | 116 ± 5 | 118 ± 7 | 107 ± 4 |
| (*RS*)-*I* | 83 ± 15 | 120 ± 10 | 116 ± 6 | 114 ± 5 | 111 ± 5 |
| | | Average blood flow in *a. femoralis*, ml/min | | | |
| (*S*)-*I* | 28 ± 8 | 115 ± 5 | 111 ± 11 | 105 ± 7 | 99 ± 6 |
| (*R*)-*I* | 30 ± 5 | 117 ± 5 | 107 ± 2 | 118 ± 6 | 101 ± 9 |
| (*RS*)-*I* | 32 ± 5 | 112 ± 7 | 114 ± 6 | 111 ± 6 | 103 ± 6 |

[a] Percentual changes of the initial value before administration ± standard deviation of the average.

due was co-distilled three times with 20 ml of water, then twice with 50 ml of ethanol and finally crystallized from ethanol. Yield 10·6 g (83·5%) of product, m.p. 162°C, $[\alpha]_D^{20}$ —87·8° (*c* 0·5, water). For $C_{10}H_{14}N_4O_4$ (254·2) calculated: 47·24% C, 5·55% H, 22·04% N; found: 47·46% C, 5·74% H, 22·34% N. UV spectrum (pH 2,7, 12): $\lambda_{max}$ 274 nm ($\varepsilon_{max}$ 10000), $\lambda_{min}$ 246 nm. $R_F$ = = 0·73 ($S_1$), 0·14 ($S_2$), 0·52 ($S_3$); theophylline 0·63 ($S_1$), 0·42 ($S_2$), 0·82 ($S_3$).

7-(*R*)-(2,3-Dihydroxypropyl)theophylline ((*R*)-*I*)

Sodium hydride (1·7 g; 70 mmol) was added to a suspension of 12·6 (70 mmol) of theophylline in 150 ml of dimethylformamide and when all the material dissolved 28·6 g (0·1 mol) of 1-O-*p*-toluenesulfonyl-2,3-O-isopropylidene-L-glycerol (see[10]) were added and the mixture stirred at 100°C for 7 h. Then the mixture was evaporated at 40°C/13 Pa and the residue extracted with 200 ml of chloroform, the material was filtered off under suction, washed with 100 ml of chloroform and the filtrate evaporated in a vacuum. To eliminate the remaining theophylline the mixture was stirred with 100 ml acetic anhydride and 1 g of 4-dimethylaminopyridine overnight at room temperature. After evaporation at 40°C/13 Pa the residue was co-distilled with two 50 ml portions of toluene under the same conditions, the residue was dissolved in 300 ml of chloroform, washed twice with 50 ml of water, dried over magnesium sulfate and evaporated *in vacuo*. The residue was chromatographed on a column of 200 g of silica gel (according to Pitra; 30—60 μm) with chloroform and the fractions containing the product ($R_F$ 0·80 in $S_2$) were combined, evaporated in a vacuum and crystallized from ethanol (light petroleum until turbid). Yield 25·7 g (63%) of 2,3-di-O-acetyl derivative of compound (*R*)-*I*, m.p. 125—126°C, $[\alpha]_D^{20}$ +73·9° (*c* 0·5, chloroform). For $C_{14}H_{18}N_4O_6$ (338·3) calculated: 49·70% C, 5·37% H, 16·56% N; found: 48·05% C, 5·49% H, 15·95% N.

A solution of 23·4 g (69·2 mmol) of this compound in 300 ml of 0·1M sodium methoxide in methanol was allowed to stand at room temperature overnight, then neutralized with dry Dowex 50 × 8 in $H^+$-form, filtered off under suction, washed with 100 ml of methanol and the filtrate was evaporated. Crystallization from ethanol gave 15·4 g (87·5%) of compound (*R*)-*I*, m.p. 159—160°C, $[\alpha]_D^{20}$ +88·2° (*c* 0·5, water), which according to chromatography in $S_1$—$S_3$ was identical with (*S*)-*I*. For $C_{10}H_{14}N_4O_4$ (254·2) calculated: 47·24% C, 5·55% H, 22·04% N; found: 47·18% C, 5·54% H, 22·34% N. The UV spectrum was identical with compound (*S*)-1.

7-(*RS*)-(2,3-Dihydroxypropyl)theophylline ((*RS*)-*I*)

This was prepared under the same conditions as the (*S*)-enantiomer in a 77·2% yield (9·8 g), m.p. 140—141°C, chromatographic data in $S_1$—$S_3$ and the UV spectrum were identical with those of compound (*S*)-*I*. $^1$H-NMR spectrum: 3·41 (s, 3 H) and 3·60 (s, 3 H) 2 N-$CH_3$; 3·61 (d, 2 H, *J* = 3) $HOCH_2$; 4·05 (m, 1 H) —CH(OH)—; 4·57 (m, 2 H) N—$CH_2$; 7·65 (s, 1 H) $C_8$—H.

Determination of the Inhibition of 3′,5′-cAMP-Phosphodiesterase

A store solution of the enzyme (Phosphodiesterase from dog heart, Boehringer, Mannheim, GFR) containing 4·5 mg of protein/ml of 0·2M Tris-HCl of pH 8·5.

*a*) Determination of $K_m$ for the cleavage of adenosine-3′,5′-cyclic phosphate: The incubation mixture contained 100 μl of substrate solution (Boehringer, Mannheim, GFR) and 25 μl of enzyme solution; the resulting concentration of the substrate was $1·2 \cdot 10^{-3}$ to $1·67 \cdot 10^{-2}$M. Incubation at 37°C lasted 30 min. After separation of the mixture in system $S_1$ the spots of the product and the substrate were eluted with 0·1M-HCl and the extent of the cleavage was calculated

from the measurement of the absorbance of both substances at 260 nm. $K_m = 3{\cdot}25 \cdot 10^{-3}$M $\pm$ $\pm$ $0{\cdot}10 \cdot 10^{-3}$M.

*b*) Determination of $K_i$ for coffein and compounds (*S*)-*I*, (*R*)-*I* and (*RS*)-*I*: The incubation mixture contained 25 µl of enzyme, 50 µl of substrate solution in 0·2M-Tris-HCl buffer of pH 8·5 ($10^{-2}$M and $2 \cdot 10^{-2}$M) and 50 µl of a solution of inhibitor in the same buffer. The resulting concentration of the substrate was $4 \cdot 10^{-3}$M and $8 \cdot 10^{-3}$M and that of the inhibitor $4 \cdot 10^{-5}$M to $8 \cdot 10^{-3}$M. Incubation at 37°C for 30 min and the determination of the extent of cleavage were carried out as under *a*). The course of the dependence $1/v \sim [i]$ was in all cases linear, the $K_i$ value was calculated from the extrapolated crossing point of the straight lines for two different substrate concentrations. The observed $K_i$ values: coffein $8{\cdot}7 \cdot 10^{-3}$M, (*S*)-*I* $1{\cdot}07 \cdot 10^{-2}$M, (*R*)-*I* $1.04 \cdot 10^{-2}$M, (*RS*)-*I* $1{\cdot}46 \cdot 10^{-2}$M (mean deviation $0{\cdot}2 \cdot 10^{-3}$M).

Determination of the Vasodilatatory Effect *in vitro*

This was carried out using isolated guinea-pig intestine (females 600—650 g) tonised with adrenaline in Krebs's cycle. From the series of 5—6 cumulative doses the concentration of the substance eliciting a half-relaxation ($ED_{50}$) was estimated. The obtained results are given in Table I.

Determination of the Parameters of the Blood Circulation in Dogs

This was carried out with dogs anesthetized with a 2% solution of pentobarbital (the weight of the experimental animals was 8—12 kg). The administration was intravenous, the substance tested (20 mg/kg) was dissolved in 10 ml of water (about 2% solution). All experimental animals obtained gradually all the three substances studied, and the sequence of application was such that two dogs first got the (*S*)-isomer, two other dogs the (*R*)-isomer and the last two dogs first the racemate of compound *I*. During the experiment the blood pressure in *a. femoralis dx.* was determined on a polygraph Galileo ECG with a tensometric head Statham P2AA, and the flow of blood in *aorta femoralis sin.* with the electromagnetic flow-meter Nycotron. The experiments were worked up using the pair *t*-test with the relevance level of 0·05. The results are given in Table II.

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