SYNTHESIS OF SOME NUCLEOLIPIDS

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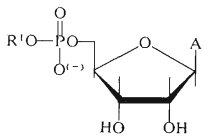
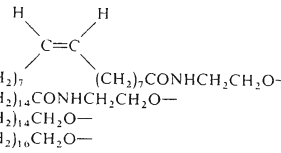
Adenosine 5'-phosphate P-(2-oleamidoethyl) ester (*I*), P-(2-palmitamidoethyl) ester (*II*), P-(1--hexadecyl ester (*III*), P-(1-octadecyl) ester (*IV*) and P-(1-adamantyl) ester (*V*) were prepared by the reaction of N,O$^{2'}$,O$^{3'}$-triacetyladenosine 5'-phosphate with appropriate hydroxy derivative in the presence of N,N'-dicyclohexylcarbodiimide followed by the action of ammonia. 2',3'-O-Ethoxymethyleneadenosine 5'-phosphate P-(2-palmitamidoethyl) ester (*IX*) was prepared by the reaction of 2-chloro-4,5-dimethyl-2-oxo-1,3,2-dioxaphosphole (*VI*) with N-(2-hydroxyethyl)palmitamide (*VII*) and 2',3'-O-ethoxymethyleneadenosine (*VIII*) followed by the action of aqueous triethylamine. Adenosine 5'-phospho-(1-decyl)amidate (*X*) and adenosine 5'-phospho--(1-pentadecyl)amidate (*XI*) were prepared by the action of 1-decylamine and 1-pentadecylamine resp. on adenosine 5'-phosphate in the presence of N,N'-dicyclohexylcarbodiimide. The compounds *I*—*VIII* are not degraded by shake venom diesterase. The inhibition of the activity of adenylate cyclase is dependent on the length of the aliphatic chain involved.

In previous papers, the effects of some esters of adenosine 5'-phosphate with lipoid hydroxy compounds (adenosine nucleolipids) on the activity of enzymes of cyclic AMP system[1,2] and on the lipolysis[3] were studied. The present paper describes the synthetic approach to these compounds and demonstrates the influence of their structural features on adenylate cyclase inhibition.

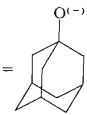
The majority of adenosine 5'-phosphate esters was prepared using the diimide phospho diester synthesis[4]. Pyridinium salt of N,O$^{2'}$,O$^{3'}$-triacetyladenosine 5'-phosphate was reacted with two equivalents of the hydroxy compound in the presence of N,N'-dicyclohexylcarbodiimide. The reactions were terminated by the addition of a small amount of water and then diluted with ether which precipitated the N,N'-dicyclohexylurea. Filtrates were evaporated and then treated with methanolic ammonia to remove the protecting acetyl groups. There is to be mentioned here that the starting experiments in nucleolipid field[1,2] were performed on esters of adenosine 5'-phosphate with 1',2'-dipalmitoyl and 1',2'-dioleylglycerol. The synthetic approach, however, did not exclude the possibility of isomerisation and therefore the work was continued using stable P-esters of aliphatic alcohols or N-acylaminoethanol. Another approach to the synthesis of nucleolipids was used in the synthesis of 2',3'--O-ethoxymethylene derivative *IX*. According to Ramirez and coworkers[5,6], 2-chloro-

-4,5-dimethyl-2-oxo-1,3,2-dioxaphosphole[7] was treated successively with N-(2-hydroxyethyl)palmitamide and 2',3'-O-ethoxymethyleneadenosine in the presence of triethylamine. The phosphoamidate analogs of nucleolipids *X* and *XI* were prepared by heating of adenosine 5'-phosphate with appropriate amine and N,N'-dicyclohexylcarbodiimide according to Moffatt and Khorana[8].

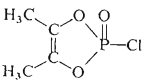




I, $R^1 = CH_3(CH_2)_7CH{=}CH(CH_2)_7CONHCH_2CH_2O-$
II, $R^1 = CH_3(CH_2)_{14}CONHCH_2CH_2O-$
III, $R^1 = CH_3(CH_2)_{14}CH_2O-$
IV, $R^1 = CH_3(CH_2)_{10}CH_2O-$

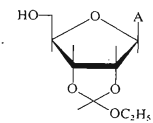


V, R^1 =



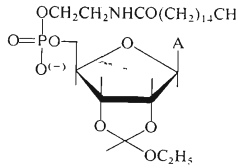
VI

1. $CH_3(CH_2)_{14}CONHCH_2CH_2OH$ *VII*



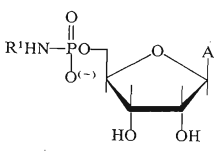
2.

VIII



IX

3. $(C_2H_5)_3N + H_2O$



X, $R^1 = CH_3(CH_2)_8CH_2-$
XI, $R^1 = CH_3(CH_2)_{13}CH_2-$

The isolation and purification of the products was dependent on their properties. Substance containing shorter chain (*V*) could be isolated by ion exchange chromato-

graphy on DEAE-cellulose column in water because their solutions could be easily evaporated. Long chains containing nucleolipids with strong detergent properties could be isolated on DEAE cellulose in 90% aqueous ethanol as was exemplified in the preparation of *IX*. Compounds *I* and *II* were isolated by chromatography on silica gel columns in methanol–chloroform systems. By this procedure nucleolipids were eluted in form of water soluble mixed salts with calcium, magnesium and sodium, according to results of Nielsen[9,10] who found an exchange between the cations of phospholipids and those in the silicic acid absorbent. Small quantities of nucleolipids *III* and *IV* were isolated and purified by preparative paper chromatography in S1 and isolated by lyophilisation.

Hydrophobic residues change the properties of these phospho diesters in a manner that they are not degraded by snake venom phosphodiesterase. This interesting finding indicates hydrophobic interaction of the aliphatic chain with the enzyme.

Adenosine nucleolipids exhibit the inhibitory effect on the activity of adenylate cyclase[1,2], which was estimated by the procedure described earlier[11]. Data in Table I strongly suggest that the inhibitory effect is directly dependent on the length of the aliphatic chain. The inhibitory effects of nucleolipids are not restricted to phosphor

TABLE I

The Effect of Nucleolipids on the Activity of Adenylate Cyclase from Rat Livers Stimulated by Glucagon or Guanylylimidodiphosphate (Gpp(NH)p)

Nucleolipid	Enzyme activity, % ± S.E.M. ($n = 3$)					
	none	100	198	316	500	1 000 μM
I	100[a]	—	75 ± 3	41 ± 2	19 ± 2	—
II	100[a]	—	16 ± 2	6 ± 1	4 ± 1	—
	100[b]	—	51 ± 2	13 ± 1	5 ± 2	4 ± 1
	100[c]	87 ± 5	—	55 ± 3	—	7 ± 1
	100[d]	70 ± 3	—	55 ± 3	—	23 ± 1
III	100[c]	90 ± 5	—	79 ± 5	—	39 ± 3
IV	100[d]	85 ± 6	—	64 ± 5	—	43 ± 3
V	100[b]	—	126 ± 7	113 ± 6	106 ± 6	107 ± 5
X	100[c]	100 ± 3	—	84 ± 5	—	72 ± 3
XI	100[c]	98 ± 5	—	80 ± 4	—	51 ± 4

[a–d] Activity of adenylate cyclase in pmol of cyclic AMP formed/mg protein/10 min (the activator used in the particular experiment is given in the bracket): [a] 440 ± 6 (1 μM glucagon); [b] 160 ± 4 (1 μM glucagon); [c] 320 ± 2 (0·05 mM Gpp(NH)p); [d] 467 ± 5 (0·1 mM Gpp(NH)p). S.E.M. standard error of mean.

diesters as the phosphoamidate analogs (*X*, *XI*) exhibit some activity in accordance to the length of their chains. On the other hand, the more compact 1 - adamantyl ester (*V*), containing the same number of carbon atoms as *X*, is without any inhibitory effect.

From the data in Table I and from our previous work[1,2] we can draw tentatively the structural requirements of nucleolipids for the inhibition of adenylate cyclase: *1*) The presence of adenosine moiety (uridine analogs are not inhibitors[2]); *2*) Aliphatic chain must have at least 10–12 carbon atoms; and *3*) The presence of negative charge on phosphorus. The study of the third requirement is, however, very difficult because of the insolubility of the analogous uncharged compounds. The flexibility of the saturated aliphatic chain seems to play an important role in the interaction of the inhibitor with adenylate cyclase; the rigid-double bond containing oleoyl derivative *I* is less active than the compound *II* with saturated aliphatic chain of the corresponding length.

EXPERIMENTAL

TLC was performed on ready-for-use Silufol UV 254 (Kavalier Glassworks, Votice, Czechoslovakia) silica gel sheets in the solvent system S1, 2-propanol–concentrated ammonia–water (7 : 1 : 2). Column chromatography was performed on macroporous silica gel (produced by Service Laboratory of this Institute).

Adenosine 5'-Phosphate P-(2-Oleamidoethyl) Ester (*I*)

Pyridinium salt of $N,O^{2'},O^{3'}$-triacetyladenosine 5'-phosphate (2·2 g; 4 mmol) is evaporated with two 20 ml portions of pyridine, N-(2-hydroxyethyl)oleamide (1·83 g; 5 mmol), N,N'-dicyclohexylcarbodiimide (4 g) and pyridine (30 ml) are added, the mixture is stirred for 1 h and then allowed to stand for 2 weeks. Water (1 ml) is added, and after 20 h, the mixture is diluted with ether (100 ml). The filtered solution is evaporated and the residue dissolved in 6M methanolic ammonia (50 ml). After 20 h the insoluble material is removed by filtration and washed with methanol (20 ml). The combined filtrates are evaporated and the residue is evaporated with two 20 ml portions of toluene and dissolved in chloroform–methanol (9 : 1) (50 ml). The solution is applied to silica gel column (4 × 40 cm) packed in chloroform. The column is eluted with chloroform–methanol 9 : 1 (500 ml) and then with chloroform–methanol 1 : 1, 20 ml fractions are collected at a 3 ml/min flow rate. Two UV-absorbing peaks are eluted with the second system. The first one affords water insoluble substance R_F (S1) 0·73 and was not further investigated. The second one affords water soluble salt of *I* (1·42 g; 47%) R_F (S1) 0·70. Molecular weight 749 as determined from the absorbance at 260 nm. The ratio C : N : P from elemental analysis is 29·8 : 6·1 : 1, theory 30 : 6 : 1.

Adenosine 5'-Phosphate P-(2-Palmitamidoethyl) Ester (*II*)

The compound was prepared analogously to *I* in the form of mixed salt. R_F (S1) 0·69. Molecular weight as determined from the absorbance at 260 nm 645. The ratio C : N : P from elemental analysis is 27·4 : 5·9 : 1, theory 28 : 6 : 1.

Adenosine 5'-Phosphate P-(1-Hexadecyl) Ester (*III*) and
Adenosine 5'-Phosphate P-(1-Octadecyl) Ester (*IV*)

Both compounds were prepared analogously to *I* and isolated as ammonium salts by preparative paper chromatography in S1 using 6 sheets of Whatman 3 MM for 1 mmol of starting nucleotide. R_F (S1) 0·62 and 0·64, respectively.

Adenosine 5'-Phosphate P-1-Adamantyl Ester (*V*)

Pyridinium salt of N,O$^{2'}$,O$^{3'}$-triacetyladenosine 5'-phosphate (2 mmol) is evaporated with two 20 portions of pyridine, 1-adamantol (4 mmol), N,N'-dicyclohexylcarbodiimide (2 g) and pyridine (10 ml) are added and the mixture allowed to stand for 2 days. Water (1 ml) is added, followed (after 10 min) by concentrated aqueous ammonia (10 ml). After 20 h, the mixture is extracted by cyclohexane, evaporated to 10 ml volume, filtered and applied to a column (500 ml; 1 : 10) of DEAE-cellulose (HCO_3^-). The elution is performed by linear gradient of triethylammonium hydrogen carbonate buffer (pH 7·5; 4 l; 0→0·15M). The UV-absorbing peak, eluted by 0·07M buffer is evaporated, the residue evaporated with two 20 ml portions of ethanol, dissolved in water and chromatographed on 6 sheets of Whatman 3 MM paper in S1. UV-Absorbing bands R_F 0·69 are eluted with 1% ammonia and lyophilised. Yield 300 mg (24%) of ammonium salt of *II*, R_F (S1) 0·62, E_{Up} 0·29. Molecular weight 635, as determined from the absorbance at 260 nm. For $C_{20}H_{31}N_6O_7P.7H_2O$ (624·7) calculated: 38·45% C, 7·11% H, 13·45% N, 4·96% P; found: 39·01% C, 6·91% H, 13·71% N, 4·66% P.

2',3'-O-Ethoxymethyleneadenosine 5'-Phosphate P-(2-Palmitamidoethyl) Ester (*IX*)

A suspension of N-(2-hydroxyethyl)palmitamide (6 g; 20 mmol) in chloroform (100 ml) and triethylamine (3 ml; 21·5 mmol) is cooled to 0°C and 2-chloro-4,5-dimethyl-2-oxo-1,3,2-dioxaphosphole (2·3 ml; 21 mmol) is added under stirring. After 1 h at 0°C and 20 h at 20°C the mixture is cooled to —10°C and a solution of 2',3'-O-ethoxymethyleneadenosine (7·4 g; 23 mmol) and triethylamine (6·4 ml; 46 mmol) in dimethylformamide (50 ml) is added. After 3 days the volatiles were evaporated, the dimethylformamide solution was diluted with water (50 ml) and triethylamine (10 ml) and pyridine (30 ml) are added. The mixture is stirred for 40 h, neutralized with carbon dioxide and diluted with ethanol to 1 000 ml. After 1 h standing, the mixture is filtered and filtrate applied to a column (2 l) of DEAE cellulose (HCO_3^-) equilibrated in 90% aqueous ethanol. The column is washed with 90% ethanol (2 l) and then with linear gradient of triethylammonium hydrogen carbonate in 90% ethanol (4 l; 0→0·05M). The gradient elution is interrupted at 0·035M buffer and the elution continued with this concentration till the end of the peak. The eluate is evaporated, the residue evaporated with three 100 ml portions of ethanol and dried under diminished pressure. Yield 7·4 g (42%) of semisolid triethylammonium salt of *IX*, R_F (S1) 0·70. Molecular weight as determined from the absorbance at 260 nm 785.

Adenosine 5'-Phospho-(1-decyl)amidate (*X*)

A mixture of adenosine 5'-phosphate (1 mmol), water (2 ml), tert-butanol (10 ml), 1-decylamine (5 mmol) and N,N'-dicyclohexylcarbodiimide is refluxed for 10 h. Water (8 ml) is added and, after 20 h, dicyclohexylurea is filtered off and washed with 50% aqueous ethanol. The filtrate is evaporated and the residue chromatographed on 6 sheets of paper Whatman 3 MM in S1. The UV-absorbing bands (R_F 0·80) are eluted with 50% aqueous 1-propanol, the eluate evaporated and dissolved in 50% ethanol (20 ml). Ammonium Dowex-50 (5 ml), is added, the mixture

stirred for 1 h and filtered. The filtrate is evaporated, the residue evaporated with two 10 ml portions of toluene and dried under diminished pressure. Yield 450 mg of ammonium salt. R_F (S1) 0·73. For $C_{20}H_{35}N_6O_7P.NH_3$ (503·5) calculated: 19·47% N, 6·15% P; found: 19·35% N, 6·02% P.

Adenosine 5′-Phospho-(1-pentadecyl)amidate (*XI*)

The compound was prepared analogously to *X*. R_F((S1) 0·85.

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