SOME DOPAMINERGICALLY ACTIVE ESTERS OF N-[(5*R*,8*S*,10*R*)-6-PROPYL-8-ERGOLINYL]CARBAMIC ACID WITH CANCEROGENICITY MANIFESTATIONS*

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Received March 17th, 1987

Esters of N-[(5*R*,8*S*,10*R*)-6-propyl-8-ergolinyl]carbamic acid *I*—*VI* were prepared from (5*R*,8*S*,-10*R*)-6-propyl-8-ergolinecarboxylic acid hydrazide (*VIII*) *via* the azide *IX* and isocyanate *X* or *via* (5*R*,8*S*,10*R*)-6-propyl-8-aminoergoline (*XI*). Some of the prepared esters exhibited strong dopaminergic activity in the neuro-endocrine region; some tests indicate possible cancerogenicity and mutagenicity of the ethyl ester *II*.

In our previous papers[1-3] we have shown that replacement of methyl group in the position 6 of some ergoline derivatives by an ethyl, and particularly n-propyl group increases substantially their prolactin-secretion inhibiting effect. Since some N-(6-methylergolinyl)carbamates[4] show a significant central dopaminergic activity (which is reflected *inter alia* in the mentioned prolactin-secretion inhibitory activity), it was of interest to see in what way the biological properties of the mentioned compounds would be modified by introducing a propyl group into the position 6. We prepared and pharmacologically studied the N-[(5*R*,8*S*,10*R*)-6-propyl-8-ergolinyl]carbamates *I*—*VI*.

The compounds *I*—*IV* and *VI* were obtained by treatment of (5*R*, 8*S*, 10*R*)-6-propyl-8-ergolinyl isocyanate (*X*) with the corresponding alcohols at about 80°C (method *A*). The compound *X* was prepared *in situ* by reaction of the starting hydrazide of (5*R*, 8*S*, 10*R*)-6-propyl-8-ergolinecarboxylic acid[5] (*VIII*) with nitrous acid, followed by decomposition of the formed azide *IX* in boiling tetrachloromethane. The tert-butyl ester *V* and benzyl ester *VI* were prepared by acylation of (5*R*, 8*S*, 10*R*)-6-propyl-8-aminoergoline (*XI*) with di-tert-butyl dicarbonate and benzyl chloroformate, respectively, in dimethylformamide in the presence of diisopropylethylamine (method *B*). The yields and physico-chemical properties of the carbamates *I*—*VI* are given in Table I. The starting aminoergoline *XI* was obtained by Curtius degradation of azide *IX* in boiling 0·1M-HCl, analogously as the known (5*R*, 8*S*, 10*R*)-6-methyl-8-aminoergoline[6].

* Part LXXII in the series Ergot Alkaloids; Part LXXI: Collect. Czech. Chem. Commun., in press.

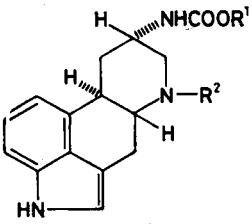
TABLE I

Esters of N-[(5*R*,8*S*,10*R*)-6-propyl-8-ergolinyl]carbamic acid

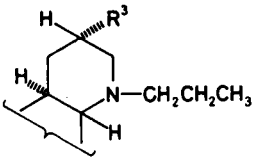
Compound[a] R^1	Yield, % (method)	M.p., °C (solvent)[b]	$[\alpha]_D^{20}$ (*c* 0·2)	Formula (mol. w.)	Calculated/found		
					% C	% H	% N
I	73	100—105	+19·0	$C_{19}H_{25}N_3O_2$	69·70	7·70	12·83
CH_3	(*A*)	S1		(327·4)	69·13	7·81	12·27
II	88	106—110	+11·2	$C_{20}H_{27}N_3O_2$	70·34	7·97	12·31
CH_3CH_3	(*A*)	S1		(341·5)	70·35	7·90	12·01
III	47	65—68	+11·2	$C_{21}H_{29}N_3O_2$	70·95	8·22	11·82
$CH_2CH_2CH_3$	(*A*)	S2		(355·5)	70·14	8·30	11·92
IV	47	70—72	+1·9	$C_{22}H_{31}N_3O_2$	71·51	8·46	11·37
$(CH_2)_3CH_3$	(*A*)	S2		(369·5)	71·11	8·11	11·54
V	68	169—171	0	$C_{22}H_{31}N_3O_2$	71·51	8·46	11·37
$C(CH_3)_3$	(*B*)	S3		(369·5)	71·77	8·65	10·90
VI	53 61	59—61	−7·2	$C_{25}H_{29}N_3O_2$	74·41	7·24	10·41
$CH_2C_6H_5$	(*A*) (*B*)	S2		(403·5)	74·17	7·43	9·88

[a] Spectral data for compounds *I*—*VI*: UV spectra (methanol), λ_{max}, nm (log ε); IR spectra (KBr) $\tilde{\nu}$, cm^{-1}; ^1H NMR spectra (deuteriochloroform, tetramethylsilane as standard). *I*: 292 (3·72), 281 (3·80), 275·5 (3·78); 3 380, 3 290 (NH), 1 750 (ester), 1 610, 1 500 (arom. + double bonds); 8·10 bs, 1 H (indole NH), 6·70—7·40 m, 4 H (arom. H), 5·82 bd, 1 H (NHCO), J = 8·0 Hz, 4·10 bm, 1 H ($C_{(8)}$—H), 3·75 s, 3 H ($COOCH_3$), 0·93 t, 3 H ($CH_2CH_2CH_3$). *II*: 292 (3·72), 281·5 (3·80), 276 (3·79); 3 350, 3 280 (NH), 1 710 (ester), 1 500, 1 610 (arom. + double bonds); 8·15 bs, 1 H (indole NH), 6·60—7·20 m, 4 H (arom. H), 5·60 m, 1 H (NHCO), 4·10 bq, 2 H (CH_2CH_3), 1·21 t, 3 H (CH_2CH_3), 0·85 bt, 3 H ($CH_2CH_2CH_3$). *III*: 292 (3·71), 281 (3·83), 575·5 (3·81); 3 360, 3 280 (NH), 1 710 (ester), 1 500, 1 610 (arom. + double bonds); 8·20 bs, 1 H (indole NH), 6·60—7·20 m, 4 H (arom. H), 5·20 bd, 1 H (NHCO), 4·00 bm, 3 H ($C_{(8)}$—H + $CH_2CH_2CH_3$), 1·50 m, 5 H (2× CH_2CH_2 + $C_{(9)}$—H), 0·90 m, 6 H (2× $CH_2CH_2CH_3$). *IV*: 292 (3·73), 281·5 (3·80), 276 (3·79); 3 360, 3 290 (NH), 1 710 (ester), 1 500, 1 610 (arom. + double bonds); 8·10 bs, 1 H (indole NH), 6·60—7·20 m, 4 H (arom. H), 5·71 bd, 1 H (NHCO), 4·05 t, 1 H ($C_{(9)}$—H) and 4·05 bm, 2 H ($CH_2(CH_2)_2CH_3$), 0·90 m, 6 H (2× CH_3). *V*: 292 (3·71), 282 (3·80), 277 sh (3·78), 224 (4·42); 3 420, 3 280 (NH), 1 690 (ester), 1 500, 1 620, 1 640 (arom. + double bonds); 8·20 bs, 1 H (indole NH), 6·60—7·20 m, 4 H (arom. H), 5·50 bd, 1 H (NHCO), J = 8·0 Hz, 4·00 bm, 1 H ($C_{(8)}$—H), 1·40 m, 2 H ($CH_2CH_2CH_3$), 1·40 m, 1 H ($C_{(9)}$—H), 1·40 s, 9 H ($C(CH_3)_3$), 0·85 t, 3 H ($(CH_2)_2CH_3$); J = 6·5 Hz. *VI*: 292 (3·74), 281 (3·83), 275 sh (3·81); 3 360, 3 300 (NH), 1 710 (ester), 1 490, 1 500, 1 620 (arom. + double bonds); 8·00 bs, 1 H (indole NH), 7·30 s, 5 H (C_6H_5), 6·50—7·20 m, 4 H (arom. H), 5·81 bd, 1 H (NHCO), J = 8·0 Hz, 5·10 s, 2 H ($CH_2C_6H_5$), 4·02 bm, 1 H ($C_{(8)}$—H), 1·20—1·70 m, 3 H ($C_{(9)}$—H + $CH_2CH_2CH_3$), 0·85 def. t, 3 H (CH_3). [b] Crystallized from: S1 benzene–cyclohexane, S2 ethanol–water, S3 acetone–hexane.

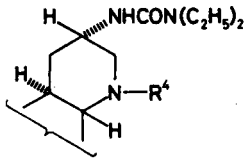
The pharmacological evaluation of compounds *I*–*VI* was predominantly aimed at their dopaminergic effect on the endocrine system. Thus, we studied the antinidation effect on fertilized rat ova[7,8] and, with selected compounds, also inhibition



I - *VI*, R^1 see Table I, R^2= $CH_2CH_2CH_3$
VII, R^1= CH_2CH_3, R^2= CH_3



VIII, R^3= $CONHNH_2$
IX, R^3= CON_3
X, R^3= NCO
XI, R^3= NH_2



XII, R^4= CH_3
XIII, R^4= $CH_2CH_2CH_3$

of lactation in lactating rats[7,9,10], the effect on prolactinemia in adult oestrogenized female rats[11] and stimulation of the ovary growth. The latter activity was measured by increase of the mean ovary weight in hemicastrated female rats, on ten days' application of the tested compound[12]. For the ethyl ester *II* we also determined its acute toxicity after intravenous administration in mice. Since compound *II* contains an ethylcarbamate group, we also studied its cancerogenic activity indicated by *in vitro* effect on a diploid human embryonic lung culture[13] (mainly cell transformations, *i.e.* the loss of contact inhibition and of original fibroblastoid morphology) and by mutagenic effect on microorganisms in a modified Ames test[14,15] on histidine-auxotrophic strains of *Salmonella typhi murium in vitro.*

The results of dopaminergic activity and acute toxicity tests on the most active compound *II*, compared with the values for the known ethyl 6-methyl-8-ergolinylcarbamate (*VII*), the clinically used 1-[(5*R*, 8*S*, 10*R*)-6-methyl-8-ergolinyl]-3,3-diethylurea[16,17] (*XII*, terguride) or its by order of magnitude more potent 6-propyl analogue *XIII* (proterguride)[3], are given in Table II. In accord with our previous findings[1-3], introduction of a propyl group into the position 6 in ergolinylcarbamates led to compounds of an order of magnitude higher inhibitory effect on prolactin

TABLE II

Dopaminergic activity and acute toxicity of ethylcarbamate *II*, compared with known ergoline derivatives *VII*, *XII*, and *XIII*

Compound	Antinidation[a] mg/kg	Antilactation[b] mg/kg	Prolactinemia[c] mg/kg		Ovary stimulation[d] mg/kg (%)	Acute toxicity[e] mg/kg
			3 h	9 h		
VII	0·27	—	—	—	—	—
XII	0·1—0·3	0·22 (0·15—0·39)	0·0089	0·045	0·04 (171)	75 (62—106)
XIII	0·010—0·015	0·010 (0·006—0·018)	0·0008	0·0051	0·002 (197)	37 (31—48)
II	0·02	0·015 (0·010—0·031)	—	0·001	0·002 (185)	11·4 (9·0—13·7)

[a] Minimum dose preventing intrauterine nidation of fertilized ova in all (100%) rats in the group after a single-dose intragastric administration on the 5th day after copulation (checked by the presence of sperms in a vaginal smear); the number of pregnant animals compared with the controls (groups of 8 animals) was followed. [b] Calculated mean effective dose (ED_{50}) which, on daily intragastric administration to lactating rats from the 5th to 8th post-delivery day reduced the mean daily weight increase of their 6 sucklings to 50% compared with the controls. [c] Calculated interpolated single intragastric dose reducing prolactinemia to a half of that found for the control group 3 or 9 h after administration to adult females castrated 13 days before the experiment and oestrogenized with 0·03 mg/kg of oestradiol dipropionate (Agofollin, Spofa) 24 hours before the application; the serum prolactin level was determined by a radioimmunoassay using the double antibody method. [d] The dose applied for 10 days, starting by the day of operation, and the mean weight of the remaining ovary on the 11th day after one-side ovariectomy of adult female rats, given as % of the ovary weight in the untreated hemicastrated control group. [e] Mean lethal dose (LD_{50}) in mice (with its confidence limits at $p = 0·95$); intravenous administration into the tail vein (group of 8 males).

TABLE III

Mutagenic effect of ethylcarbamate *II* in a modified Ames test

Compound[a]	Dose[b] µg	Activation[c]	*Salmonella typhi murium*				
			TA 1 535	TA 1 537	TA 1 538	TA 98	TA 100
II	1 000	+	1 581	21	26	45	1 362
		−	1 274	12	5	31	1 224
		+	1 560	19	135	81	2 205
		−	1 045	9	11	20	1 211
	100	+	31	13	13	79	187
		−	425	8	8	54	524
		+	497	17	36	36	166
		−	894	10	8	13	602
	10	+	11	13	13	18	105
		−	33	5	5	20	157
		+	14	15	26	23	126
		−	158	7	7	9	158
	1	+	12	13	12	30	132
		−	13	5	7	16	127
		+	20	16	23	15	137
		−	36	4	5	8	145
AF	10	+	−	−	>2 000	>500	>1 000
		+	−	−	>2 000	>2 000	>1 500
AA	40	−	−	40	−	−	−
		−	−	40	−	−	−
EMS	5	−	>1 000	−	−	−	−
		−	>1 000	−	−	−	−
DMSO	0·1 ml	+	9	19	13	31	91
		−	10	9	6	26	89
		+	19	13	20	28	137
		−	16	9	13	15	125

[a] As standard mutagenes were tested: 2-aminofluorene (AF) on TA 1 538, TA 98, and TA 100 strains, 9-aminoacridine (AA) on TA 1537 strain, and ethyl methanesulfonate (EMS) on TA 1 535 strain; dimethyl sulfoxide (DMSO) as control. [b] The compounds were tested *in vitro* on histidine-auxotrophic strains of *Salmonella typhi murium* after metabolic activation with rat liver microsomes as well as without activation, repeatedly in the given doses (*per* 2.5 ml of the medium) in dimethyl sulfoxide solution. Three dishes were prepared for each strain and dose and, after incubation at 37°C for 48 h, the number of mutants was counted. In the controls, only the solvent was applied. The Table lists the mean number of mutants counted on 3 dishes; values from two experiments are given. The strains TA 1535 and TA 100 are geneticaly identical and differ only in the presence of plasmide pKM 101 in the latter strain (resistance to ampicilin). [c] + Test after metabolic activation of the tested compound, − test without metabolic activation.

secretion and ovary stimulating effect, comparable with that of *XIII*. The acute toxicity of *II* is about three times higher than that of *XIII* but its value is negligible compared with the effective dose. The lower aliphatic esters *I* and *III* were about as effective as compound *II* (Antilactation ED_{50} 0·01 and 0·05 mg/kg, respectively) whereas the higher esters *IV*, *V* and *VI* were substantially less active (ED_{50} 0·12, 0·31, and 0·21 mg/kg, respectively).

In a cell culture, the behaviour of compound *II* suggests cancerogenicity: At concentration 146 μmol/l some cells were transformed after 24 h and on the 5th day more than 50% of the cell population were transformed; at concentration 29 μmol/l there were still some cells transformed after 72 h, their amount rising to 5% after five days. This effect is comparable with the cancerogenic activity of ethyl N-ethylcarbamate which in a parallel experiment at the same concentration (146 μmol/l) induced a significant cell transformation after 24 h and transformation of more than 50% cells after 5 days. This finding was confirmed by a modified Ames test whose results are given in Table III. The mutagenic effect of compound *II* has been proven on histidine-auxotrophic strains of *Salmonella typhi murium* TA 1335 and TA 100; a significant increase of the mutants was found after metabolic activation of the compound (1 000 μg dose) as well as without metabolic activation (doses 1 000 and 100 μg/2·5 ml medium). The observed cell transformations in the culture and the mutagenic effect in the Ames test make *II* a cancer suspect compound. Since the unsubstituted ethylurethane is a known cancerogene, we may justifiably suppose that the mentioned effect is caused by the ethylcarbamate group in the molecule of *II*. This experience cautions us to be careful in the choice and synthesis of compounds containing verified cancerogenic groups.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Analytical samples were dried over phosphorus pentoxide at 20—100°C/30 Pa to a constant weight. Specific rotations were determined on a Perkin–Elmer 141 polarimeter and refer to solvent-free substances. Ultraviolet spectra were taken on a Pye Unicam SP 8 000 spectrophotometer in methanol (concentration (w/v) 0·004 and 0·0008%), IR spectra were recorded in KBr pellets on a Perkin–Elmer 577 instrument. Proton NMR spectra were obtained with a Tesla BSC 487 (80 MHz) spectrometer, concentration (w/v) about 10%; δ values are given in ppm. Purity of the compounds was checked by thin-layer chromatography on reflex foils of silica gel with a luminiscent indicator (Silufol UV_{254}, Kavalier, Czechoslovakia) in the system chloroform–ethanol–triethylamine (90 : 10 : 5) and/or benzene–dioxane–ethanol–triethylamine (50 : 40 : 10 : 5); spots were detected by UV light at 254 nm or by spraying with 0·5% solution of 4-dimethylaminobenzaldehyde in cyclohexane followed by exposure to hydrochloric acid fumes. Column chromatography was performed on silica gel Merck Kieselgel 60, No. 7734 or on Silpearl (Kavalier).

Ethyl N-[(5*R*,8*S*,10*R*)-6-Propyl-8-ergolinyl]carbamate (*II*) (Method *A*)

A solution of sodium nitrite (5 ml of 1M-$NaNO_2$; 5 mmol), followed by 0·2M-HCl (27·5 ml; 5·5 mmol), was added dropwise at 2—5°C to a stirred solution of hydrazide *VIII* (1·56 g; 5 mmol)

in 0·1M-HCl (50 ml; 5 mmol). After 15 min the mixture was made alkaline with 1M-$NaHCO_3$ (20 ml) and the separated azide *IX* was taken up in tetrachloromethane. The organic extract was dried over sodium sulphate at +5°C and refluxed under exclusion of moisture until nitrogen evolution ceased (about 15 min). The thus-obtained hot solution of the isocyanate *X* was refluxed with ethanol (10 ml) for half hour, the solvents were evaporated and the crude product (1·5 g; 88%) was purified by column chromatography on silica gel with chloroform as eluant. The combined homogeneous product fractions were taken down under reduced pressure and the residue was crystallized (see Table I). Esters *I*, *III*, *IV*, and *VI* were prepared in an analogous manner using methanol, 1-propanol, 1-butanol or benzyl alcohol, respectively, instead of ethanol.

Tert-butyl N-[(5*R*,8*S*,10*R*)-6-Propyl-8-ergolinyl]carbamate (Method *B*)

Diisopropylethylamine (0·14 g; 1·1 mmol) was added under stirring to a suspension of 8-amino-ergoline *XI* (0·27 g; 1 mmol) in dimethylformamide (6 ml), the mixture was cooled to 5—10°C and di-tert-butyl dicarbonate (0·24 g; 1·1 mmol) was added. The homogeneous mixture was set aside for 3 h, poured into ice-cold water (25 ml) and the product was taken up in chloroform. After drying over sodium sulphate and evaporation of the solvent, the crude product (0·35 g) was purified by chromatography on a column of silica gel in chloroform and crystallization (see Table I). The benzyl ester *VI* was prepared analogously using benzyl chloroformate (0·19 g; 1·1 mmol) instead of di-tert-butyl dicarbonate.

(5*R*,8*S*,10*R*)-6-Propyl-8-aminoergoline (*XI*)

A solution of *IX* in dilute hydrochloric acid, obtained from *VIII* (1·56 g; 5 mmol) as described for *II*, was added dropwise to a stirred boiling 0·1M-HCl (100 ml). After refluxing for 2 min, the mixture was cooled to room temperature, adjusted to pH 8—9 with 1M-NaOH and the product was extracted with chloroform. The organic extract was dried over sodium sulphate, the solvent was evaporated and the crude product (1·25 g; 92%) was crystallized from methanol, m.p. 223—225°C; $[\alpha]_D^{20}$ −42·2° (*c* = 0·2, pyridine). For $C_1H_{23}N_3$ (269·4) calculated: 75·76% C, 8·61% H, 15·60% N; found: 75·33% C, 8·79% H, 15·43% N. UV spectrum (in methanol), λ_{max}, nm (log ε): 293 (3·74), 283 (3·83), 277·5 inf. (3·80), 225·5 (4·54). IR spectrum (KBr), $\tilde{\nu}$, cm^{-1}: 3 340, 3 400, 3 140 (NH, NH_2); 1 615, 1 605, 1 565 (arom.). ^1H NMR (hexadeuteriodimethyl sulfoxide): 7·90 bs, 1 H (indole NH); 6·60—7·20 m, H (arom. H); 1·62 dt, 1 H ($C_{(9)}$—H), *J*(H-8 eq, H-9 ax) = 4·8 Hz, *J*(H-9 ax, H-10 ax) = *J*(H-9 ax, H-9 eq) = 13·3 Hz; 1·40 m, 2 H ($CH_2CH_2CH_3$), 0·90 t, 3 H ($CH_2CH_2CH_3$).

Elemental analyses were performed by Mrs J. Komancová in the Analytical Department of this Institute (Dr J. Körbl, Head), UV and IR spectra were measured by Dr J. Vachek, NMR spectra by Dr J. Holubek, both of the Physico-Chemical Department of this Institute (Dr B. Kakáč, Head). We are indebted to the National Hormone and Pituitary Program, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Baltimore, Maryland, U.S.A., for the rat prolactin assay kits.

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Translated by M. Tichý.