ERGOT ALKALOIDS. XXXVIII.*

SOME AMIDES OF D-6-METHYL-8-ERGOLIN(I)YLACETIC ACID

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Received August 3rd, 1970

The azide method was applied to the preparation of simpler amides of D-6-methyl-8-ergolin(I)-ylacetic acid *I*—*VIII*. The acid amide *I* was also prepared by reductive cleavage of ammonia from the hydrazide of the acid using Raney nickel. Particularly the amide *I* displayed a pronounced antifertility and antilactating effect on rats.

The present communication describes the synthesis of the simpler amides of D-6-methyl-8-ergolin(I)ylacetic acid *I*—*VIII* (Table I) and some results of an informative study of selected compounds as to their antifertility and antilactating effect on rats. The study was stimulated by the discovery of these effects with D-6-methyl-8-cyanomethylergolin(I) (see ref.[1,2]) and with the amide of D-6-methyl-8-ergolin(I)ylacetic-acid (*I*) prepared as the first compound of this particular series.

The amides *I*—*VI* and *VIII* were prepared by the azide method, using the reaction of the relatively stable hydrochloride of the azide of D-6-methyl-8-ergolin(I)ylacetic acid[1] with excess aqueous ammonia, or with excess corresponding amine required also for releasing the azide base from its salt and for binding the hydrazoic acid formed during the reaction. In the case of amide *VII* we replaced the excess (+)-2-amino-1-butanol with triethylamine. In the case of amide *VIII* we worked in a mixture of triethylamine with ether using excess glycine ethyl ester. The crude products were purified mostly first by chromatography on a column of silica gel or directly by crystallization or sublimation. The amide *I* was prepared also by reductive cleavage of ammonia from the hydrazide of D-6-methyl-8-ergolin(I)ylacetic acid with Raney nickel, in a medium of aqueous ethanol[3]. The amide *I* was characterized also in the form of normal tartrate and maleate, the amide *III* as hydrogen maleate.

* Part XXXVII: Československ. farm., in press.

The testing of the compounds as to their antifertility effects was done with pregnant Wistar rats (7 experimental and 7 control animals), using a *p.o.* application of an aqueous solution of their salts. At this place, we present some data on amides *I*, *III* and *V*. More detailed data on the results of this study will be published elsewhere. Amide *I*: With all the experimental animals pregnancy was interrupted after a single dose of 1 mg normal tartrate of the base per animal (some 5 mg/kg) administered between the 1st and the 7th day after mating. The same effect was achieved on applying 80 μg preparation (about 400 μg/kg) but only on the 6th or 7th day after mating and after daily application for the first five days of 25 μg per animal (total of about 625 μg/kg). For comparative purposes we applied normal tartrate of the amide of D-6-methyl-8-ergolenylacetic acid (ref.[4]) in a dose of 80 μg per animal on the 6th or 7th day after mating and found it to have no effect. The acute LD_{50} of normal tartrate of the amide *I* for mice upon *i.v.* administration was found to be 93 mg/kg, upon oral administration about 1 g/kg. The antifertility effect of amide *I* can be prevented by prolactin. The preparation increases the enhanced secretion of gonadotropins observed in rats after unilateral ovariectomy and, in proportion to the dose, reversibly decreases or practically stops lactation in rats. Amide *III*, applied as hydrogenmaleate, and amide *V*, applied as normal tartrate, given on five days of the first seven days after mating, prevented pregnancy in all the experimental animals at a daily dose of 1 mg and 0·5 mg per animal, respectively.

EXPERIMENTAL

The melting points (frequently decomposition) were determined in Kofler's block and have not been corrected. Samples for analysis were dried at 100°C/0·2 Torr. The values of specific rotation correspond to compounds free of crystal solvent. The composition of the fraction obtained by column chromatography on silica gel (according to Pitra and Štěrba[5], grain size 60—120 μ) as well as of other preparations was investigated by chromatography on paper impregnated with formamide, with an addition of ammonium formate, using chloroform as the mobile phase, or in a system of 1-butanol–acetic acid–water (4 : 1 : 5), or else on thin layers of silica gel G (according to Stahl, Merck), using chloroform–acetone (2 : 1). The compounds were detected on paper by their UV fluorescence after previous illumination with sunlight, or by their reaction with Ehrlich's reagent. On the thin layers they were detected on the basis of the purple-blue colour after spraying with a 10% solution of *p*-toluenesulfonic acid in methanol and after heating to 50°C (for analogous detection of ergot alkaloids see ref.[6]).

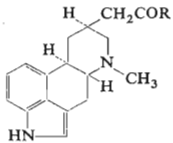
Amides of D-6-Methyl-8-ergolin(I)ylacetic Acid *I*— *VIII*

Unless stated otherwise, azide hydrochloride of D-6-methyl-8-ergolin(I)ylacetic acid (*IX*), used for the reactions, was prepared from the hydrazide of the same acid *X* (ref.[1]) and freshly filtered to leave a minimum residue of moisture. The solvents used for the crystallization of the products and some physical properties of the coumpounds may be found in Table I.

Amide I: *a*) A mixture of azide hydrochloride *IX* prepared from 3·5 g hydrazide *X* and 28 ml concentrated aqueous ammonia was left standing under occasional shaking for 24 h at 20°C. After adding 44 ml water the product was filtered, washed with water (2·9 g, 87·5%) and, after

TABLE I

Amides of D-6-Methyl-8-ergolin(I)ylacetic Acid



Compound	R	Formula (m.w.)	M.p., °C (solvent)	$[\alpha]_D^{20}$ (*c*, pyridine)	Calculated/Found % C	% H	% N
I[a]	NH_2	$C_{17}H_{21}N_3O$ (283·4)	237—239·5 (methanol)	−83·7° (0·38)	72·04 72·32	7·47 7·74	14·83 14·64
II	NHC_2H_5	$C_{19}H_{25}N_3O$ (311·4)	198—200 (methanol)	−74·5° (0·68)	73·27 72·78	8·09 8·45	13·50 13·39
III[b]	$N(C_2H_5)_2$	$C_{21}H_{29}N_3O$ (339·5)	237—240	−80·0° (0·4)	74·29 73·99	8·61 8·99	12·38 12·10
IV	$NH(CH_2)_5CH_3$	$C_{23}H_{33}N_3O$ (367·5)	165—167 (acetone)	−68·6° (0·74)	75·16 75·16	9·05 9·26	11·43 11·56
V		$C_{22}H_{29}N_3O$ (351·5)	196—198 (methanol)	−68·0° (0·46)	75·17 74·79	8·32 8·68	11·95 11·91
VI		$C_{22}H_{29}N_3O$ (351·5)	204—207 (acetone)	−71·0° (0·65)	75·17 74·83	8·32 8·32	11·95 11·89
VII		$C_{21}H_{29}N_3O_2$ (355·5)	217—221 (acetone–benzene)	−82·5° (0·4)	70·95 71·13	8·22 8·52	11·82 11·89
VIII	$NHCH_2CO_2C_2H_5$	$C_{21}H_{27}N_3O_3$ (369·5)	203—205 (chloroform–benzene–hexane)	−70·0° (0·4)	68·26 68·02	7·37 7·40	11·38 11·20

[a]Normal tartrate of amide *I*: m.p. 254—257°C (methanol); contains 3 molecules of crystal solvent which are lost on drying at 100°C/0·2 Torr; for $(C_{17}H_{21}N_3O)_2.C_4H_6O_6.3\ CH_3OH$ (812·9) calculated: 11·82% CH_3OH; found: 11·89% CH_3OH. $[\alpha]_D^{20}$ −28·2° (*c* 0·2, water). For $(C_{17}H_{21}.N_3O)_2.C_4H_6O_6$ (716·8) calculated: 63·37% C, 6·75% H, 11·73% N; found: 63·33% C, 6·74% H, 12·05% N.

Normal maleate of amide *I*: on heating it melts at 136—138°C, then partly, crystallizes and melts again at 250—256°C (methanol); it contains 2·5 molecules of crystal solvent which are lost on drying at 100°C/0·1 Torr. For $(C_{17}H_{21}N_3O)_2.C_4H_4O_4.2{\cdot}5\ CH_3OH$ (762·9) calculated: 10·5% CH_3OH; found: 10·77% CH_3OH; $[\alpha]_D^{20}$ −32·4° (*c* 0·2, water). For $(C_{17}H_{21}N_3O)_2$.

dissolving in 94 ml methanol, it was chromatographed on a column of 20 g silica gel, using the same solvent for elution. The combined fractions containing the almost homogeneous amide *I* were purified by crystallization.

b) A mixture of 2·0 g hydrazide *X*, 15 ml suspension of freshly prepared Raney nickel (containing roughly 0·067 mol hydrogen) decanted with 95% ethanol and 1 000 ml 95% ethanol was refluxed for 1 h. After filtration of the catalyst, the filtrate was evaporated to dryness, the residue (1·40 g, 98%) was dissolved in methanol and purified by chromatography as shown under *a*), then by crystallization from methanol. In its m.p. (237—239°C), specific rotation ($[\alpha]_D^{20}$ −84° (*c* 0·2, pyridine)) and in its behaviour during chromatography on paper and on thin layers the preparation corresponds to the same compound prepared under *a*). For $C_{17}H_{21}N_3O$ (283·4) calculated: 72·04% C, 7·47% H, 14·83% N; found: 71·99% C, 7·61% H, 14·83% N.

Normal tartrate and maleate of the base were prepared by using equimolar amounts of both components, in a medium of methanol.

Amide II: Hydrochloride of azide *IX*, prepared from 1·5 g hydrazide *X* was mixed at 0°C with 10·5 g ethylamine and the solution left to stand for 4 days at constant temperature. The precipitated product was filtered, washed with water and combined with another fraction obtained by saturating the combined filtrates with sodium chloride (a total of 1·2 g; 77%). The solution of the product in methanol was purified by chromatography just as in the case of amide *I* and then by crystallization.

Amide III: Hydrochloride of the azide *IX*, prepared from 1·4 g hydrazide *X*, was placed into 7·1 g diethylamine cooled to 0°C, the mixture was set aside for 48 h at 20°C, the precipitated product was filtered and washed with water. Another fraction of the product was obtained by diluting the combined mother liquors with water, extracting the product with chloroform and distilling off the volatile fractions in water-pump vacuum. Combined fractions of the compound (1·15 g, 72%) were purified by sublimation at 200°C/0·5 Torr.

Hydrogenmaleate of the base was prepared by using equimolar amounts of both components in methanol.

Amides IV *and* VI: Hydrochloride of the azide *IX*, prepared from 1·5 g hydrazide *X*, was placed into 7·6 g n-hexylamine, or 8·6 g piperidine, the mixture was agitated until dissolving of the solid, then set aside for 60 h at 20°C. In the case of amide *IV*, the mixture was poured into 250 ml of a mixture of benzene and ether (2 : 1), filtered and freed of the volatile fractions by distillation in water-pump vacuum. The viscous residue was shaken with 250 ml water and the solid product was filtered (1·1 g, 59·5%) and purified by crystallization. In the case of amide *VI* the precipitated product was filtered and washed with water. Another fraction of the product was obtained by dilution of the mother liquor with water, extraction of the product with chloroform and distillation of the volatile fractions. The combined products (0·9 g, 51%) were purified by crystallization.

Amide V: Hydrochloride of the azide *IX* prepared from 1·9 g hydrazide *X* was placed at 20°C into 3·5 g cyclopentylamine and the mixture left to stand under occassional agitation, for 24 h at constant temperature. After pouring the mixture into 150 ml water the product was extracted with chloroform, the extract freed of the volatile fractions by distillation in water-pump vacuum, the residue dissolved in methanol and purified chromatographically as in the case of amide *I* (1·4 g, 63%) and then by crystallization.

$C_4H_4O_4$ (682·8) calculated: 66·84% C, 6·79% H, 12·31% N; found: 66·64% C, 6·89% H, 12·59% N. [b]Hydrogen maleate of amide *III*: m.p. 168—171°C (methanol), $[\alpha]_D^{20}$ −35° (*c* 0·4, water). For $C_{21}H_{29}N_3O.C_4H_4O_4$ (455·5) calculated: 65·90% C, 7·30% H, 9·23% N; found: 65·54% C, 7·37% H, 8·96% N.

Amide VII: 1·4 g hydrochloride of the azide *IX*, dried over phosphorus pentoxide at 0·5 Torr, was placed in a mixture of 1·2 g (+)-2-amino-1-butanol and 2·2 g triethylamine cooled to −10°C. After 24 h of standing at 20°C the mixture was diluted with 45 ml water and left standing overnight. The precipitated product was filtered, washed with water (0·85 g, 51%), dissolved in 20 ml of a mixture of chloroform and ethanol (5 : 2), the solution filtered through a column of 5 g silica gel which was then washed with the same mixture of solvents. The combined filtrates were freed of the volatile fractions by distillation, the residue was boiled with 5 ml acetone and, after adding 10 ml benzene, the mixture was set aside at −5°C. The precipitated product was washed with benzene.

Amide VIII: Hydrochloride of the azide *IX* prepared from 1·0 g hydrazide *X* was mixed with 2·0 g hydrochloride of glycine ethyl ester and 10 g triethylamine, the mixture was triturated and, after adding 30 ml ether, left to stand for 48 h at 20°C. The product was filtered, washed with ether and water, dissolved in chloroform and the solution filtered through a column of 3 g alumina of activity III−IV which was then washed with a mixture of chloroform and acetone. A warm solution of the residue (0·35 g, 28%) of the combined filtrates in 8 ml chloroform was poured into a mixture of 15 ml benzene and 5 ml hexane and the mixture left to crystallize at −5°C.

The analyses were carried out by Mr K. Havel and Mrs M. Komancová under the direction of Dr J. Kŏrbl in the analytical department of this institute; paper chromatography was done by Mrs M. Jelínková and Dr K. Macek, the LD_{50} of the amide was determined by Dr I. Podvalová, all of this Institute.

REFERENCES

1. Semonský M., Kucharczyk N.: This Journal *33*, 577 (1968).
2. Řežábek K., Semonský M., Kucharczyk N.: Nature *221*, 667 (1969).
3. Ainsworth C.: US-Pat. 2 756 235.
4. Troxler F., Stadler P. A.: Helv. Chim. Acta *51*, 1060 (1968).
5. Pitra J., Štěrba J.: Chem. listy *56*, 544 (1962).
6. Leemann H. G., Weller H.: Helv. Chim. Acta *43*, 1359 (1960).

Translated by A. Kotyk.