New compounds with cytotoxic and antitumour effects VIII.* Preparation and *in vitro* activity of some pregnane derivatives on leukemia P 388 cells

†[a]A. VASSOVÁ, [a]Z. VOTICKÝ, [b]J. FUSKA, and [b]A. FUSKOVÁ

[a]*Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava*

[b]*Department of Biochemical Technology, Slovak Technical University, CS-812 37 Bratislava*

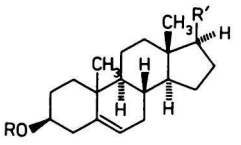
Received 14 November 1985

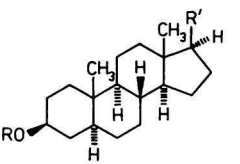
Eight new and six already known derivatives of pregnane were synthesized and their cytostastic *in vitro* activities on leukemia P 388 cells were tested. The most effective substance of this series was found to be (20*R*)--amino-3β-acetoxy-5α-pregnane (*Va*), which preferentially inhibited in a 50—100 μg cm^{-3} concentration the incorporation of [^{14}C]-labelled uridine and L-valine in all P 388 fractions and stopped proliferation of cells *in vitro*.

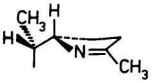
Получено восемь новых и шесть уже описанных производных прегнана и проверялось их цитостатическое действие на клетки лейкемии Р 388 на опыте. Обнаружено, что самым действенным веществом из данной серии является (20*R*)-амино-3β-ацетокси-5α-прегнан (*Va*), который в концентрации 50—100 мкг см$^{-3}$ ингибировал, главным образом, инкорпорацию [^{14}C] меченного уридина и L-валина во всех фракциях Р 388 и прекращал пролиферацию клеток на опыте.

A relatively good cytostatic effect [1] of the alkaloid veracintine — (20*S*,22*S*)--3β-hydroxy-20-(2-methyl-1-pyrrolin-5-yl)pregn-5-ene (*I*) [2—4] on the leukemia P 388 cells stimulated us to prepare derivatives of 5,6-dehydropregnane and 5α-pregnane substituted by a five-membered heterocycle in the side chain similarly as encountered with *I*. A suitable and available starting material for the proposed synthesis appeared to be 3β-hydroxy-5,6-dehydropregnan-20-one (*II*) and its acetate (*IIa*). An attempt to condense these compounds with a proper reactant possessing active methylene group failed and therefore, the corresponding oximes were prepared in about 100 % yields. The Bouveault—Blanc reduction of oximes with sodium metal in 2-propanol afforded a mixture of (20*R*) — *III* and (20*S*) — *IIIa* amino enantiomers in $r_n \approx 4:3$. Catalytic hydrogenation of the oximes on Adams catalyst in acetic acid yielded the respective 5,6-dihydro-20-amino derivatives of 5α-pregnane *IV*, *IVa*, *V*, and *Va*. Catalytical

* For Part *VII* see Ref. [1].





I R = H R′ = 

II R = H R′ = H_3C–C(=O)–

IIa R = Ac R′ = H_3C–C(=O)–

III R = H R′ = H_3C–C(H)(NH_2)– (20*S*)

IIIa R = H R′ = H_3C–C(H)(NH_2)– (20*R*)

VI R = H R′ = H_3C–C(H)–N(CO)(CO)

IV R = H, *V* R = Ac R′ = H_3C–C(H)(NH_2)–

IVa R = H, *Va* R = Ac R′ = H_3C–C(H)(NH_2)–

VII R = H, *VIII* R = Ac R′ = H_3C–C(H)–N(CO)(CO) (3′,4′; 20)

IX R = H, *XI* R = Ac R′ = H_3C–C(H)–N(CO)(CO)–C(CH$_3$)=

X R = H, *XII* R = Ac R′ = H_3C–C(H)–N(CO)(CO)–C(=CH$_2$)–

XIII R = CO–$(CH_2)_2$COOH R′ = H_3C–C(H)–N(CO)(CO)

hydrogenation proceeded almost quantitatively, the $c((20R) - III) : c((20S) - IIIa) \approx 1$ 1. Condensation of the above-mentioned amines with dicarboxylic anhydrides (succinic, citraconic, itaconic) in pyridine led to compounds having a five-membered heterocyclic ring attached to C-20 of the steroid backbone. The (20*S*)-amino derivatives reacted at room temperature to give compounds *VI*—*XII*, the (20*R*) epimer *IIIa* reacted at an enhanced temperature only to furnish *XIII*; at higher temperature also the 3β-hydroxy group was acylated.

Structures of some model compounds were backed by spectral (mass, IR, ^{1}H NMR) means, the already known (20*R*) and (20*S*) amines were distinguished according to their physicochemical properties. Compounds *IX* to *XII* are very little soluble in alcohols and therefore, no optical rotation could be taken. Some discrepancies in melting point and optical rotation data between our products and those reported in the literature might be due to higher optical purity.

Table 1

Incorporation inhibition (*I*) of [^{14}C]-labelled precursors in P 388 cells *in vitro*

Compound	*I*/%			
	Adenine	L-Valine	Thymidine	Uridine
I	14.4	72.7	50.3	31.9
II	23.7	+ 5.4	53.8	13.1
III	3.0	4.6	1.5	26.0
IIIa	20.7	30.7	38.2	45.4
IV	3.8	16.4	19.6	7.3
IVa	14.9	38.1	43.4	7.0
V	43.6	61.3	65.8	52.4
Va	89.8	94.5	85.2	97.0
VI	25.0	22.6	39.5	15.0
VII	24.6	17.6	58.0	40.0
VIII	57.6	42.4	46.5	33.7
IX	8.0	8.1	4.6	6.4
X	0	16.2	8.7	1.5
XI	50.4	72.4	50.5	69.3
XII	39.7	60.0	32.9	39.3
XIII	18.9	3.6	21.2	24.2

Concentration of compounds tested in dimethyl sulfoxide: 100 μg cm^{-3}

The prepared substances and the starting *II* were tested on cytostatic effect by the method described in [3]. Results are listed in Table 1. The high percentage of incorporation inhibition of labelled L-valine, thymidine, and uridine in a standard experiment (100 μg of (20*R*)-amino-3β-acetoxy-5α-pregnane (*Va*) in 1 cm^3 of dimethyl sulfoxide) prompted us to test this substance even at lower

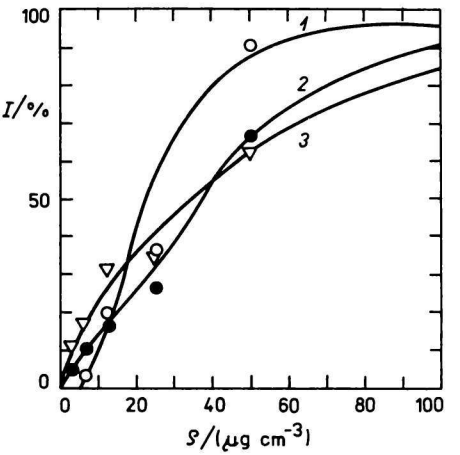


Fig. 1. Concentration dependence of incorporation inhibition (*I*) of [^{14}C]-labelled L-valine (*1*), uridine (*2*), and thymidine (*3*) in P 388 cells by (20*R*)-amino-3β-acetoxy-5α-pregnane (*Va*) *in vitro*.

concentrations ($\varrho/(\mu g\,cm^{-3})$ = 50, 25, 12.5, 6.25, and 3); the results revealed the inhibition of incorporation to be greater than 60 % also in a half concentration (Fig. 1). The most effective of the compounds prepared was found to be (20*R*)--amino-3β-acetoxy-5α-pregnane (*Va*) and (20*S*)-citraconimido-3β-acetoxy-5α--pregnane (*XI*); the methyl group of the latter is located at the double bond of the heteroring as in veracintine (*I*).

The (20*R*)-amino derivatives are more effective than their (20*S*) counterparts. The C-5—C-6 double bond of pregnane only little influenced the effect. Acetylation of the hydroxyl group in 3β-position resulted in a water more soluble product, which is more active than the starting derivative.

Experimental

Melting points were determined on a Kofler micro hot-stage, optical rotation of chloroform solutions (unless stated otherwise) was measured with Perkin—Elmer, model 141, spectrophotometer, the IR spectra (KBr technique) and electron-impact mass spectra were recorded with Perkin—Elmer, model 457, and AEI-MS 902 (70 eV, 100 μA) apparatuses, respectively. The ^{1}H NMR spectra of deuteriochloroform solutions (internal reference tetramethylsilane) were run with a Bruker AN-300 instrument operating at 300 MHz. Alumina with gypsum and silica gel (Woelm TLC) coated plates, dried at room temperature for 24 h were employed for thin-layer chromatography in following solvent systems: S_1: chloroform—ethanol, $r_{l'}$ = 9 : 1; S_2: benzene—ethanol, $r_{l'}$ = 85 : 15; S_3: benzene—ethanol, $r_{l'}$ = 9 : 1.

(20S)-Amino- (III) and (20R)-amino-5-pregnen-3β-ol (IIIa)

Sodium metal (2 g) was successively added to a boiling solution of 3β-hydroxy-5-pregnen-20-one oxime (438 mg; 1.3 mmol) in 2-propanol (40 cm^3) and the mixture was refluxed under exclusion of moisture for 3 h. Water (100 cm^3) was added to the cooled mixture, which was acidified with H_2SO_4 (2 mol dm^{-3}) to pH = 6 and then basified with ammonia. The product was extracted with chloroform (5 × 100 cm^3), the organic layer was washed with water, dried, and the solvent was distilled off. Since the reduction proceeded to approximately 50 % as monitored by thin-layer chromatography, the product was chromatographed through a silica gel column (silica gel (Lachema, Brno) L 40—100). Elution with benzene—ethanol, $r_{l'}$ = 96 : 4 afforded the starting material with benzene—ethanol, $r_{l'}$ = 7 : 3 the (20*R*)-amino-5-pregnen-3β-ol (*IIIa*), m.p. = 215 °C (benzene—methanol, $r_{l'}$ = 2 : 1), [α] (578 nm, 21 °C) = −52° (ϱ = 0.55 g dm^{-3}, ethanol), R_f = 0.51 (S_1); Ref. [5] gives m.p. = 218 °C, [α] (578 nm) = −76.2°; Ref. [6]: m.p. = 170—172 °C, [α] (578 nm) = −60.4°. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1050 (ν(C—O)), 1380 and 1440 (δ(CH_3)), 1600 (δ(NH)). Elution with benzene—ethanol, $r_{l'}$ = 6 : 4 and work-up yielded (20*S*)-amino-5-pregnen-3β-ol (*III*),

m.p. = 162 °C (methanol), $[\alpha](578\,\text{nm}, 22\,°\text{C}) = -42.8°$ ($\varrho = 0.64\,\text{g dm}^{-3}$, methanol), $R_f = 0.20$ (S_1); Ref. [5]: m.p. = 171—173 °C, $[\alpha](578\,\text{nm}) = -65.6°$. IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$ 1050 (ν(C—O)), 1380 and 1440 ($\delta(CH_3)$), 1600 (δ(NH)).

(20S)-Amino- (IV) and (20R)-amino-5α-pregnan-3β-ol (IVa)

3β-Hydroxy-5-pregnen-20-one oxime (200 mg; 0.6 mmol) dissolved in glacial acetic acid (15 cm^3) was hydrogenated over Adams catalyst (100 mg) at room temperature for 12 h. The mixture was worked up in a routine way to furnish two isomers, which were separated on an alumina-packed column (6 g, Reanal, activity grade II). First fractions obtained by elution with benzene—ethanol, $r_V = 95:5$ gave (20*R*)-amino-3β-hydroxy--5α-pregnane (*IVa*), m.p. = 176 °C (benzene—ethanol, $r_V = 95:5$), $[\alpha](578\,\text{nm}, 21\,°\text{C}) = 7.5°$ ($\varrho = 0.6\,\text{g dm}^{-3}$, ethanol), $R_f = 0.34$ (S_2); Ref. [5]: m.p. = 176—177 °C, $[\alpha](578\,\text{nm}) = 3°$ IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$: 1050 (ν(C—O)), 1380 and 1440 ($\delta(CH_3)$), 1600 (δ(NH)). Further fractions contained (20*S*)-amino-3β-hydroxy-5α-pregnane (*IV*), m.p. = 172—173 °C, $[\alpha](578\,\text{nm}, 21\,°\text{C}) = 12°$, ($\varrho = 0.5\,\text{g dm}^{-3}$, ethanol), $R_f = 0.11$ (S_2); Ref. [5]: m.p. = 173 °C, $[\alpha](578\,\text{nm}) = 13°$ IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$: 1050 ($\nu$(C—O)), 1380 and 1440 ($\delta(CH_3)$), 1600 (δ(NH)).

(20S)-Amino- (V) and (20R)-amino-3β-acetoxy-5α-pregnane (Va)

3β-Acetoxy-5α-pregnan-20-one oxime (440 mg; 1.3 mmol) dissolved in glacial acetic acid (15 cm^3) was hydrogenated over Adams catalyst (150 mg) at room temperature for 12 h. The catalyst was removed, the solution was diluted with water ($r_V = 1:4$), made alkaline and the product was taken into chloroform (5 × 20 cm^3). The dried extract was evaporated and the residue was chromatographed through an alumina-packed column (15 g, Reanal, activity grade II). (20*R*)-Amino-3β-acetoxy-5α-pregnane (*Va*) was obtained by elution with benzene—ethanol, $r_V = 99:1$; m.p. = 146—147 °C (ethyl acetate), $[\alpha](578\,\text{nm}, 21\,°\text{C}) = -2°$ ($\varrho = 0.58\,\text{g dm}^{-3}$, methanol), $R_f = 0.47$ (S_3); Ref. [5]: m.p. = 146—147 °C, $[\alpha](578\,\text{nm}) = -16.7°$ IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$ ($CHCl_3$): 1030 (ν(C—O)), 1250 (ν(C—O—C)), 1600 (δ(NH)), 1715 (ν(CO)). (20*S*)-Amino-3β-acetoxy--5α-pregnane (*V*) was washed out with benzene—ethanol, $r_V = 95:5$; m.p. = = 149—150 °C (dichloroethane), $[\alpha](578\,\text{nm}, 20\,°\text{C}) = -8°$ ($\varrho = 0.57\,\text{g dm}^{-3}$), $R_f = 0.16$ (S_3); Ref. [5]: m.p. = 117—118 °C, $[\alpha](578\,\text{nm}) = -10.5°$. IR spectrum, $\tilde{\nu}/\text{cm}^{-1}$ ($CHCl_3$): 1030 (ν(C—O)), 1250 (ν(C—O—C)), 1600 (δ(NH)), 1730 (ν(CO)).

(20S)-Alkanedicarboximidopregnanes VI—XIII

Pyridine solution of the respective pregnane (0.15 mmol) was treated with the proper dicarboxylic anhydride (0.15 mmol) in the same solvent at room temperature for 24 h, the solvent was distilled off under diminished pressure and the crude product was crystallized

from ethanol, compound *XIII* from chloroform. Yields of this condensation varied within 70 and 85 %.

(20S)-Succinimido-5-pregnen-3β-ol (VI): from (20*S*)-amino-3β-hydroxy-5-pregnene (*III*, 49 mg) in pyridine (5 cm^3) and succinic anhydride (15 mg) in pyridine (1.5 cm^3); m.p. = 188—190 °C (methanol), $[\alpha]$(578 nm, 21 °C) = −39° ($CHCl_3$—C_2H_5OH, $r_V = 1:2$, $\varrho = 3\ g\ dm^{-3}$). Mass spectrum, *m/z*: 399 ($M^{+\bullet}$), 384 (*M* − 15), 381 (*M* − 18), 366, 301 (*M* − 98), 267, 215, 44. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1050 (ν(C—O)), 1380 and 1440 (δ(CH_3)), 1620 (ν(C=C)), 1715 (ν(CO)).

(20S)-Succinimido-5α-pregnan-3β-ol (VII): from (20*S*)-amino-3β-hydroxy-5α-pregnane (*IV*, 50 mg) in pyridine (6 cm^3) and succinic anhydride (15 mg) in pyridine (1.5 cm^3); m.p. = 204—205 °C (methanol), $[\alpha]$(578 nm, 22 °C) = 5° ($CHCl_3$—C_2H_5OH, $r_V = 2:1$, $\varrho = 5\ g\ dm^{-3}$). Mass spectrum, *m/z*: 401 ($M^{+\bullet}$), 386 (*M* − 15), 383 (*M* − 18), 368, 303, 302, 287, 44. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1050 (ν(C—O)), 1650 (ν(N—CO)), 1715 (ν(CO)).

(20S)-Succinimido-3β-acetoxy-5α-pregnane (VIII): from (20*S*)-amino-3β-acetoxy-5α-pregnane (*V*, 52 mg) in pyridine (12 cm^3) and succinic anhydride (15 mg) in pyridine (1.5 cm^3); m.p. = 221—222 °C (ethanol), $[\alpha]$(578 nm, 20 °C) = −5.2° ($CHCl_3$, $\varrho = 6\ g\ dm^{-3}$). Mass spectrum, *m/z*: 443 ($M^{+\bullet}$), 428 (*M* − 15), 383 (*M* − 60), 345, 44, 43. ^1H NMR spectrum, δ/ppm: 0.77 (s, 3H, C-18—H_3), 0.90 (s, 3H, C-19—H_3), 1.20 (d, 3H, C-21—H_3, $J = 8$ Hz), 2.07 (s, 3H, C-3—$OCOCH_3$), 2.62 (q, 4H, C-3′, C-4′—$2H_2$, $J = 10$ Hz), 4.00 (m, 1H, C-17—H), 4.70 (m, 1H, C-3—H). IR spectrum, $\tilde{\nu}/cm^{-1}$: 1030 (ν(C—O)), 1250 (ν(C—O—C)), 1715 (ν(CO)), 1730 (ν(O—CO—CH_3)).

(20S)-Citraconimido-5α-pregnan-3β-ol (IX): from (20*S*)-amino-3β-hydroxy-5α-pregnane (*IV*, 50 mg) in pyridine (5 cm^3) and citraconic anhydride (17 mg) in pyridine (2 cm^3); m.p. = 218—219 °C (methanol). Mass spectrum, *m/z*: 413 ($M^{+\bullet}$), 398 (*M* − 15), 395 (*M* − 18), 380 (*M* − 33), 303. ^1H NMR spectrum, δ/ppm: 0.74 (s, 3H, C-18—H_3), 0.86 (s, 3H, C-19—H_3), 1.23 (d, 3H, C-21—H_3, $J = 9$ Hz), 2.07 (s, 3H, $OCOCH_3$), 2.11 (d, 2H, C′-3—H_2, $J = 6$ Hz), 4.03 (m, 1H, C-17—H), 4.68 (m, 1H, C-3—H), 6.45 (t, 1H, C′-4—H). IR spectrum, $\tilde{\nu}/cm^{-1}$: 1040 (ν(C—O)), 1625 (ν(C=C)), 1700 (ν(CO)).

(20S)-Itaconimido-5α-pregnan-3β-ol (X): from (20*S*)-amino-3β-hydroxy-5α-pregnane (*IV*, 287 mg; 0.9 mmol) in pyridine (10 cm^3) and itaconic anhydride (101 mg; 0.9 mmol) in pyridine (5 cm^3); m.p. = 223—224 °C (methanol). Mass spectrum, *m/z*: 413 ($M^{+\bullet}$), 398 (*M* − 15), 395 (*M* − 18), 380 (*M* − 33), 44. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1045 (ν(C—O)), 1630 (ν(C=C)), 1700 (ν(CO)).

(20S)-Citraconimido-3β-acetoxy-5α-pregnane (XI): from (20*S*)-amino-3β-acetoxy-5α-pregnane (*V*, 52 mg) in pyridine (5 cm^3) and citraconic anhydride (17 mg) in pyridine (2 cm^3); m.p. = 210 °C (ethanol). Mass spectrum, *m/z*: 455 ($M^{+\bullet}$), 395 (*M* − 60), 343, 44, 43. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1030 (ν(C—O)), 1250 (ν(C—O—C)), 1630 (ν(C=C)), 1710 (ν(CO)), 1730 (ν($OCOCH_3$)).

(20S)-Itaconimido-3β-acetoxy-5α-pregnane (XII): from (20*S*)-amino-3β-acetoxy-5α-pregnane (*V*, 361 mg; 1 mmol) in pyridine (10 cm^3) and itaconic anhydride (112 mg; 1 mmol) in pyridine (5 cm^3); m.p. = 204 °C (ethanol). Mass spectrum, *m/z*: 455 ($M^{+\bullet}$), 395 (*M* − 60), 343, 44, 43. IR spectrum, $\tilde{\nu}/cm^{-1}$: 1030 (ν(C—O)), 1250 (ν(C—O—C)), 1630 (ν(C=C)), 1710 (ν(CO)), 1730 (ν($OCOCH_3$)).

(20R)-Succinimido-3β-(3-carboxypropanoyloxy)-5α-pregnane (XIII)

Succinic anhydride (15 mg) in pyridine (1 cm^3) was added to a solution of (20*R*)-amino-3β-hydroxy-5α-pregnane (*IVa*, 50 mg) in pyridine (3 cm^3). The mixture was heated on a steam bath for 1 h, the solvent was distilled off under diminished pressure and the residue was crystallized from chloroform; m.p. = 227 °C, $[\alpha](578\ nm, 22\ °C) = 32.5°$ ($CHCl_3$—C_2H_5OH, $r_V = 2:1$, $\varrho = 3.7\ g\ dm^{-3}$). Mass spectrum, *m/z*: 501 ($M^{+\bullet}$), 403 ($M - 98$), 383 ($M - 118$), 44. ^1H NMR spectrum, δ/ppm: 0.67 (s, 3H, C-18—H_3), 0.80 (s, 3H, C-19—H_3), 1.08 (d, 3H, C-21—H_3, $J = 6$ Hz), 2.5 (q, 4H, $(CH_2)_2$), 2.7 (dd, 4H, C′-3, C′-4—$2H_2$), 2.96 (m, 1H, C-3—H), 4.02 (m, 1H, C-17—H). IR spectrum, $\tilde{\nu}/cm^{-1}$: 1030 (ν(C—O)), 1700 (ν(CO)).

References

1. Fuska, J., Fusková, A., Vassová, A., and Votický, Z., *Neoplasma 28*, 709 (1981).
2. Tomko, J., Brázdová, V., and Votický, Z., *Tetrahedron Lett. 1971*, 3041.
3. Vassová, A., Votický, Z., Tomko, J., and Ahond, A., *Collect. Czechoslov. Chem. Commun. 41*, 2964 (1976).
4. Pavelčík, F. and Tomko, J., *Chem. Zvesti 37*, 145 (1983).
5. Van De Woude, G. and Van Hove, L., *Bull. Soc. Chim. Belg. 76*, 566 (1967).
6. Lucas, R. A., Dickel, D. F., Dzieman, R. L., Ceglowski, M. J., Heusle, B. L., and MacPhillamy, H. B., *J. Amer. Chem. Soc. 82*, 5688 (1960).

Translated by Z. Votický