Semisynthetic analogues of Buxus alkaloids*

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3β-Acetoxy-20-oxopregna-5,16-diene reacted with methyl nitroacetate in the presence of acetic acid and ammonium acetate to afford 3β-acetoxy-16α-(1-nitro-1-methoxycarbonylmethyl)-20-oxopregn-5-ene, 3β-acetoxy-16α-nitromethyl-20-oxopregn-5-ene, (24*S*)-3β-acetoxy-22-aza-23-oxo-24-nitro-16,24-cyclochola-5,17-diene, and 3β-acetoxy-22-aza-23-hydroxy-24-nitro-16,24-cyclochola-5,17,22,24-tetraene. The latter originated from the former in the presence of alumina.

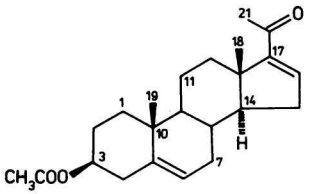
Реакция 3β-ацетокси-20-оксопрегна-5,16-диена с метилнитроацетатом в присутствии уксусной кислоты и уксуснокислого аммония приводила к образованию 3β-ацетокси-16α-(1-нитро-1-метоксикарбонилметил)-20-оксопрегн-5-ена, 3β-ацетокси-16α-нитрометил-20-оксопрегн-5-ена, (24*S*)-3β-ацетокси-22-аза-23-оксо-24-нитро-16,24-циклохола-5,17-диена и 3β-ацетокси-22-аза-23-гидрокси-24-нитро-16,24-циклохола-5,17,22,24-тетраена. Последний продукт образовывался из предыдущего диена в присутствии окиси алюминия.

The interest in Buxus alkaloids has become raising after it was reported [1] that one of the major bases — cyclovirobuxine-D — exerted positive inotropic and negative chronotropic effects on isolated toad and rabbit hearts, and, in anesthetized dogs it caused a significant increase in coronary blood flow. Aiming to prepare biologically active compounds we synthesized some model substances related to Buxus alkaloids.

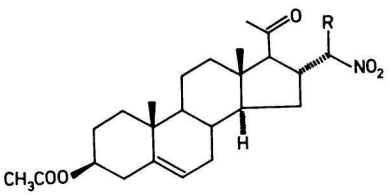
Steroidal ketones having an α,β-unsaturated D-ring appeared to be suitable starting material for this project; thus 3β-acetoxy-20-oxopregna-5,16-diene (*I*) afforded upon Michael addition of methyl nitroacetate a mixture of adducts, which yielded four products when separated by chromatography. The first of them was identified by spectral and physical methods as the expected 3β-acetoxy-16α-(1-nitro-1-methoxycarbonylmethyl)-20-oxopregn-5-ene (*II*).

This stereospecific addition introduced three additional centres of chirality into the molecule of *II*, *i.e.* to carbons C-17, C-16a, and C-16. Configuration 17*S* was deduced from the positive value $\Delta\varepsilon(285) = +0.56$ ($[\Theta]_{max} = 1858$) of the

* Part *XXIII* in the series *Buxus alkaloids*; for Part *XXII* see *Chem. Zvesti 38*, 255 (1984).

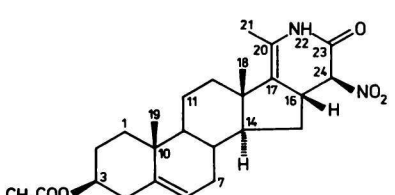


I

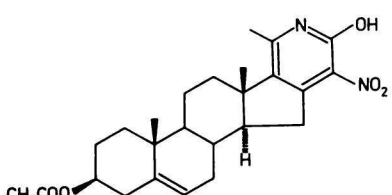


II R = $COOCH_3$
III R = H

CD spectrum; this run of the CD curve is at the same time characteristic of the α-position of the substituent at the adjacent carbon C-16 [2], which corresponds to the 16*R* configuration at the given substitution pattern. As known [3], attachment of the side chain at C-17 influences the position of signals of the neighbouring C-18 in the ^{1}H NMR spectrum. Steroids having an acetyl group at C-17 in β-position and a substituent at C-16 in α-position revealed the corresponding signal at δ/ppm $\approx$ 0.7. This signal is paramagnetically shifted (δ/ppm $\approx$ 1.0) when the respective substituents are in positions 16α and 17α. Spectrum of compound *II* displayed the signal due to C-18 at δ/ppm = 0.70 (J(16, 17) = 9.6 Hz), this being indicative of 16α, 17β orientation in line with the above-mentioned chiroptic measurements and considerations on the course of additions of steroids belonging to the 14α series. Addition to the double bond of 14β*H*-20-oxopregn-16-ene was reported to have an opposite sterical course under formation of a 16β, 17α derivative [4]. The molecule of *III* differed from the former by the loss of the methoxycarbonyl group; it originated from *II via* hydrolysis of the C-16a ester group followed by a spontaneous decarboxylation of the carboxyl group being formed. This decarboxylation was subject to the presence of a geminally bound nitro group.

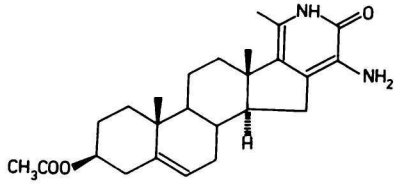


IV



V

Obviously, compound *III* could be assigned the structure of 3β-acetoxy-16α--nitromethyl-20-oxopregn-5-ene.



VI

The IR spectrum of compound *IV* lacked the band at $\tilde{\nu} = 1700\ \mathrm{cm}^{-1}$ associated with the vibration of carbonyl group bound to C-17 and accordingly, also the ^{1}H NMR spectrum did not contain the signal δ/ppm = 2.17 (CH_3CO—); instead a new signal of methylene group appeared at δ/ppm = 1.85 (J = 2.2 Hz) split into a doublet through interaction with the C-16 proton. Like shift and signal splitting of protons C-21—H are typical of pregna-5,17(20)-diene derivatives, as *e.g.* the product of Diels—Alder reaction of 20-oxo-pregna-5,16-diene with methyl vinyl ether (δ/ppm = 1.82, J = 1.5 Hz) [5, 6], or derivatives of 24-norchola-5,17(20)-dienoic acid [7]. Based on molecular formula $C_{25}H_{34}N_2O_5$ compound *IV* contained two atoms of nitrogen the first of which was embodied in a nitro group ($\nu_{as}(NO_2) = 1560\ \mathrm{cm}^{-1}$, $\nu_s(NO_2) = 1345\ \mathrm{cm}^{-1}$), the second in an amide in a 6-membered ring (ν(CO) = 1680 cm^{-1}, ν(NH) = 3350 cm^{-1}). Considering these facts a 6-methyl-3-nitro-5,6-dehydropiperidin-2-one grouping could be anticipated in this moiety of *IV*. Formation of compound *IV* can be rationalized by reaction of the adduct *II* with ammonium acetate. The C-16α configuration for this compound was proposed, since the same configuration has been proved for the adduct *II* and no other changes took place at the carbon under consideration. The magnitude of coupling constant J(16, 24) = 15.0 Hz is indicative of a *trans* arrangement of protons at C-16 and C-24 and therefore the configuration at C-24 had to be β. The last isolated compound *V* might be an artifact originating from *IV* during purification on alumina; this presumption was evidenced in an experiment in which compound *IV* dissolved in benzene was stirred with alumina at room temperature. During 2 h *IV* was quantitatively transformed into *V*. This process did not occur with silica gel. The elemental analysis of *V* showed a loss of two hydrogen atoms when compared with compound *IV*; these could stem from carbons C-16 and C-24 under aromatization of ring E. In favour of this proposal is the UV spectrum of compound *V* with its last absorption band at $\lambda = 366$ nm ($\log(\varepsilon/(\mathrm{m}^2\ \mathrm{mol}^{-1})) =$ $= 3.76$), which underwent a bathochromic shift in alkaline medium to $\lambda = 414$ nm. Similar properties were reported for *e.g.* 3-nitro-2-pyridone ($\lambda =$ $= 363$ nm ($\log(\varepsilon/(\mathrm{m}^2\ \mathrm{mol}^{-1})) = 3.87$)) [8]. The band associated with the nitro group in the IR spectrum was shifted to $\tilde{\nu} = 1515\ \mathrm{cm}^{-1}$, this being characteristic

Table 1

^{13}C NMR chemical shift data (δ/ppm) of compounds *IV*—*VI*

C	*IV*	*V*	*VI*
1	36.9^{+}	36.8	36.9^{+}
2	27.7°	27.6	27.7
3	73.7	73.6	73.8
4	38.0	38.0	38.1
5	139.7	139.9	140.0
6	121.8	121.7	122.0
7	31.3	31.3	31.6
8	31.6	30.5	30.7
9	49.7	49.8	50.0
10	36.8	36.7	36.8^{+}
11	20.8	20.7	20.9
12	36.7^{+}	35.9	36.2
13	44.0	44.7	44.6
14	55.9	56.0	56.7
15	27.6°	33.0	29.0
16	40.2	134.4	129.5
17	126.4	130.3	130.5
18	17.2	17.1	17.6
19	19.3	19.2	19.3
20	122.1	144.1	124.0
21	15.6	16.9	15.8
23	162.1	157.2	158.3
24	89.3	157.0	133.1

$^{+}$, ° in the column could be interchanged.

of nitro groups attached to double bonds. The band due to amide was observed at $\tilde{\nu} = 1600\ \mathrm{cm}^{-1}$, a value close to that of substituted 2-pyridones [9], which occurred in an enol form. The ^{1}H NMR spectrum of *V* disclosed the signal of C-21 protons at δ/ppm = 2.41 as a singlet; this value is close to that of methyl groups bound to an aromatic ring. The C-15—H signals were upfield shifted to δ/ppm = 3.05 (dd, $J(5\alpha, 16\beta) = 17.5$ Hz, $J(14\alpha, 15\alpha) = 6.4$ Hz, C-15—Hα) and δ/ppm = 2.69 (dd, $J(14\alpha, 15\beta) = 13.1$ Hz, C-15—Hβ) as a result of a double bond formation. Further arguments evidencing structures *IV* and *V* were provided by the electron impact mass spectrum. The absence of molecular radical-ion is a common feature of all afore-mentioned compounds; as known, C-3 acetoxypregnanes underwent the McLafferty rearrangement. Compound *IV* revealed the peak of ion with $m/z = 395$ (loss of HNO_2), which further eliminated the methyl radical and a neutral molecule of acetic acid (species at $m/z = 380$, 335, and 320 (parent peak)). The base peak of compound *V* was found at $m/z = 380$ $(M - CH_3COOH)^{+\bullet}$, further characteristic peaks appeared

at $m/z = 425$ $(M - CH_3)^+$ and 365 $(380 - CH_3)^+$. The presence of the ion of m/z 423 $(M - OH)^+$ indicated the existence of an enol form of the pyridone *V*. Sodium dithionate reduction of *V* led to the amine *VI*. These facts are in accordance with the data obtained by analysis of the ^{13}C NMR spectra (Table 1). Signal positions ascribed to carbons C-17 and C-20 were confirmed in all cases by the selective INEPT [10], utilizing the C-18 and C-21 methyl groups for polarization transmission. Changes in the signal positions of carbons belonging to ring E when passing from *V* to *VI* backed the change in tautomerism: the enol form prevails in compound *V*, the keto form in compound *VI*.

The arguments presented allow to assign following structures: (24*S*)-3β-acetoxy-22-aza-23-oxo-24-nitro-16,24-cyclochola-5,7-diene, 3β-acetoxy-22-aza-23--hydroxy-24-nitro-16,24-cyclochola-5,7,22,24-tetraene, and 3β-acetoxy-24-amino-22-aza-23-oxo-16,24-cyclochola-5,17-diene to compounds *IV*, *V*, and *VI*, respectively.

Experimental

Melting points were determined on a Kofler micro hot-stage, the electron impact mass spectra were recorded with a Jeol JMS 100 D apparatus at ionization energy 70 eV, the UV and IR spectra were measured with Specord UV VIS (Zeiss, Jena) and Perkin—Elmer, model 983 spectrophotometers, respectively. The ^1H and ^{13}C NMR spectra of deuteriochloroform solutions containing tetramethylsilane as the internal reference were taken with a Bruker, model AM-300 spectrometer at 300 and 75 MHz, respectively. The CD spectra were measured with a Jobin Yvon Mark III-S dichrograph. Pure substances were obtained by column chromatography on alumina (Reanal, Budapest, activity grade IV) and silica gel (Merck, activity grade V); the composition of fractions was monitored by thin-layer chromatography on alumina in solvent systems chloroform—benzene (volume ratio = 10 : 15, S_1), chloroform (S_2), and chloroform—ethanol—toluene (volume ratio = 14 : 2 : 5, S_3), detection by iodine vapours.

Reaction of 3β-acetoxy-20-oxopregna-5,16-diene with methyl nitroacetate

Solution of 3β-acetoxy-20-oxopregna-5,16-diene (*I*) (710 mg; 2 mmol), methyl nitroacetate (0.258 g; 2.3 mmol), ammonium acetate (0.60 g; 7.8 mmol) in benzene (40 cm^3) and acetic acid (2.4 cm^3) was refluxed for 7 h. The solvents were evaporated under reduced pressure and the residue dissolved in benzene was chromatographed on an alumina-packed column (100 g) by a gradient elution with benzene—chloroform. The eluates were monitored by thin-layer chromatography in solvent systems S_1 and S_2. The work-up of fraction B afforded a solid (163 mg), which was crystallized from dichloromethane—methanol (volume ratio = 10 : 1) to yield *II* (45 mg). Rechromatography of mother liquors under the same conditions furnished the second crop of

II (30 mg) and compound *III* (18 mg). Fraction D (245 mg) containing two compounds was separated by column chromatography over silica gel (120 g) using the mixture chloroform—benzene (volume ratio = 1 : 1) into compounds *IV* and *V* as seen by thin-layer chromatography in S_2. Both were separately crystallized from dichloromethane—methanol (volume ratio = 10 : 1) to give pure *IV* (68 mg) and *V* (128 mg).

3β-Acetoxy-16α-(1-nitro-1-methoxycarbonylmethyl)-20-oxo-pregn-5-ene (II): m.p. = = 194—196 °C, R_f = 0.38 (S_1), 0.95 (S_2). For $C_{26}H_{37}NO_7$ (M_r = 475.5) w_i(calc.): 65.66 % C, 7.84 % H, 2.94 % N; w_i(found): 65.52 % C, 7.67 % H, 2.90 % N. IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 1760 ν(C-16a—$COOCH_3$), 1735 ν(C-3—$OCOCH_3$), 1700 ν(C-17—$COCH_3$), 1560 $\nu_{as}(NO_2)$. ^{1}H NMR spectrum ($CDCl_3$), δ/ppm: 5.40 (m, 1H, C-5—H), 5.00 (d, 1H, C-16a—H, J(16, 16a) = 8.0 Hz), 4.62 (m, 1H, C-3—H), 3.75 (s, 3H, $COOCH_3$), 2.68 (d, 1H, C-17—H, J(16, 17) = 9.5 Hz), 2.17 (s, 3H, C-21—H), 2.05 (s, 3H, C-3—$OCOCH_3$), 1.07 (s, 3H, C-19—H), 0.70 (s, 3H, C-18—H). Mass spectrum, m/z (I_r/%): 415 (100), 400 (15), 370 (8), 358 (6), 350 (7), 338 (3), 323 (4), 295 (6).

3β-Acetoxy-16α-nitromethyl-20-oxopregn-5-ene (III): m.p. = 145—147 °C, R_f = 0.41 (S_1), 0.96 (S_2). For $C_{24}H_{35}NO_5$ (M_r = 417.5) w_i(calc.): 69.04 % C, 8.45 % H, 3.35 % N; w_i(found): 68.83 % C, 8.33 % H, 3.24 % N. IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 1723 ν(C-3—$OCOCH_3$), 1688 ν(C-17—$COCH_3$), 1555 $\nu_{as}(NO_2)$. ^{1}H NMR spectrum ($CDCl_3$), δ/ppm: 5.35 (m, 1H, C-6—H), 4.60 (m, 1H, C-3—H), 4.30 (m, 2H, C-16a—H), 3.50 (m, 1H, C-16—H), 2.50 (d, 1H, C-17—H, J(16, 17) = 8.5 Hz), 2.20 (s, 3H, C-21—H), 1.11 (s, 3H, C-19—H), 0.70 (s, 3H, C-18—H). Mass spectrum, m/z (I_r/%): 357 (100), 342 (15), 145 (7), 121 (4), 107 (3).

(24S)-3β-Acetoxy-22-aza-23-oxo-24-nitro-16,24-cyclochola-5,17-diene (IV): m.p. = = 215—217 °C, R_f = 0.05 (S_1), 0.40 (S_2). For $C_{25}H_{34}N_2O_5$ (M_r = 442.5) w_i(calc.): 67,85 % C, 7.74 % H, 6.33 % N; w_i(found): 67.69 % C, 7.57 % H, 5.99 % N. IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 3220 ν(N—H), 1730 ν(C-3—$OCOCH_3$), 1680 ν(C-23=O), 1560 $\nu_{as}(NO_2)$, 1345 $\nu_s(NO_2)$. ^{1}H NMR spectrum ($CDCl_3$), δ/ppm: 5.40 (m, 1H, C-6—H), 5.07 (d, 1H, C-24—H, J(16, 24) = 15.0 Hz), 4.60 (m, 1H, C-3—H), 3.76 (m, 1H, C-16—H), 2.05 (s, 3H, C—$OCOCH_3$), 1.85 (d, 3H, C-21—H, J(16, 21) = 2.2 Hz), 1.03 (s, 3H, C-19—H), 0.96 (s, 3H, C-18—H). Mass spectrum, m/z (I_r/%): 427 (0.5), 410 (6), 395 (10), 382 (2), 336 (19), 335 (60), 321 (35), 320 (100), 200 (10), 174 (23), 160 (25).

3β-Acetoxy-22-aza-23-hydroxy-24-nitro-16,24-cyclochola-5,17,22,24-tetraene (V): m.p. = 236—239 °C, R_f = 0.03 (S_1), 0.20 (S_2). For $C_{25}H_{32}N_2O_5$ (M_r = 440.5) w_i(calc.): 68.16 % C, 7.32 % H, 6.35 % N; w_i(found): 67.91 % C, 7.18 % H, 6.09 % N. UV spectrum (methanol), λ_{max}/nm (log {ε}/(m^2 mol^{-1})) = 218 (3.35), 366 (2.76); 0.2 mol dm^{-3} methanolic KOH: 414 (2.64). IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 3500—3400 ν(OH), 1730 ν(C-3—$OCOCH_3$), 1635 ν(C=C), 1515 $\nu_{as}(NO_2)$. ^{1}H NMR spectrum ($CDCl_3$), δ/ppm: 5.40 (m, 1H, C-5—H), 4.61 (m, 1H, C-3—H), 3.05 (dd, 1H, C-15α—H, J(15α, 15β) = 17.5 Hz, J(14α, 15α) = 6.4 Hz), 2.69 (dd, 1H, C-15β—H, J(14α, 15β) = 13.1 Hz), 2.41 (s, 3H, C-21—H), 2.04 (s, 3H, C-3—$OCOCH_3$), 1.08 (s, 3H, C-19—H), 1.03 (s, 3H, C-18—H). Mass spectrum, m/z (I_r/%): 425 (7), 411 (11), 410 (27), 381 (29), 380 (100), 366 (16), 365 (43), 364 (11), 349 (18), 335 (18), 320 (27), 207 (28).

3β-Acetoxy-24-amino-22-aza-23-oxo-16,24-cyclochola-5,17-diene (VI)

Solution of *V* (29.7 mg; 0.07 mmol) in methanol (80 cm^3) was stirred with sodium dithionate in water (20 cm^3, $\varrho = 0.025\ g\ cm^{-3}$); the mixture was kept at 40 °C for 1 h, and filtered, the filtrate was evaporated and the residue was triturated with chloroform ($3 \times 10\ cm^3$). The chloroform extracts were combined and dried, the solvent was removed and the residue was chromatographed on a preparative sorbent-coated plate in S_3. Extraction of the zone with R_f 0.64—0.54 with chloroform—methanol (volume ratio = = 10:1) and work-up afforded the title product. Yield = 16 mg (28 %), m.p. = 169—171 °C. For $C_{25}H_{34}N_2O_3$ ($M_r = 410.5$) w_i(calc.): 73.14 % C, 8.34 % H, 6.82 % N; w_i(found): 72.89 % C, 8.17 % H, 6.67 % N. IR spectrum (KBr), $\tilde{\nu}/cm^{-1}$: 3400—3300 ν(N—H), 1730 ν(C-3—$OCOCH_3$), 1660 ν(C=C). ^1H NMR spectrum ($CDCl_3$), δ/ppm: 5.42 (m, 1H, C-5—H), 4.61 (m, 1H, C-3—H), 2.53 (dd, 1H, C-15α—H, $J(15\alpha, 15\beta) =$ = 15.2 Hz, $J(14\alpha, 15\alpha) = 6.2$ Hz), 2.22 (s, 3H, C-21—H), 2.04 (s, 3H, C-3—$OCOCH_3$), 1.08 (s, 3H, C-19—H), 0.95 (s, 3H, C-18—H). Mass spectrum, *m/z* (I_r/%): 410 (0.4), 350 (100), 335 (12), 334 (16).

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