17-(*O*-(2-CARBOXYETHYL)OXIME) DERIVATIVES OF ANDROSTANES OF 3-HYDROXY-5α-, -5β-, -5-ENE AND 3-OXO-4-ENE SERIES*

Vladimir POUZAR and Ivan CERNY

Institute of Organic Chemistry and Biochemistry,
Academy of Sciences of the Czech Republic, 166 10 Prague 6, The Czech Republic

Received October 12, 1994
Accepted November 1, 1994

Androstane 17-(*O*-(2-carboxyethyl)oxime) derivatives were prepared either by the reaction of 17-keto derivatives with corresponding substituted hydroxylamine or by the addition of 17-oximino derivatives to the alkyl acrylate and subsequent hydrolysis. Oxidation of the hydroxy group in position 3 in derivatives of this type was performed either by the Oppenauer reaction, transforming 5-ene derivatives into 3-oxo-4-enes, or with Jones reagent in the case of saturated 5α- or 5β-derivatives. Configuration 17*E* in the whole series of oximes was confirmed by the ^{1}H and ^{13}C NMR spectroscopy.

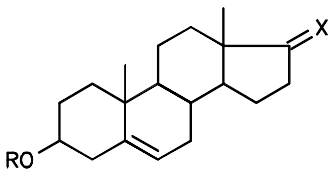
O-(2-Carboxyethyl)oximes (CEO) are the first homologues of the widely used *O*-(carboxymethyl)oxime (CMO) derivatives. Unlike the latter, routinely used in haptene conjugation forming bridge between the active part of haptene and a label or a carrying protein, CEO derivatives are referred only scarcely. Italian authors[1] described their use for a synthesis of 5-aza-4-oxaprostaglandine analogues, CEO derivatives of [3.2.0]bicycloheptanone were prepared[2], and a series of CEO derivatives of simple ketones[3] were studied for the antiinflammatory activity. In the steroid field only 17-CEO estrone derivatives are known from the patent literature[4].

As an alternative to the CMO haptens with slightly extended connecting bridge, we were firstly interested in preparations of CEO derivatives in 17-oxoandrostane series. Previous experiments revealed the stability of these derivatives toward the isomerization or transesterification reactions, which might be useful in more complex syntheses.

For the preparations of CEO derivatives from carbonyl compounds hitherto two procedures were employed. The reaction of ketone with the substituted hydroxylamine[1], i.e. (*O*-(2-carboxyethyl))hydroxylamine is the first possibility. Second one consists in the base catalyzed addition of suitable acrylic acid ester to the oxime of starting ketone and affords the corresponding ester of CEO derivative[3].

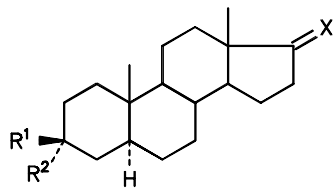
* Part CCCLXXVIII in the series On Steroids; Part CCCLXXVII: Collect. Czech. Chem. Commun. *59*, 2691 (1994).

By the latter procedure, adding ethyl acrylate to the steroidal 17-oximino derivatives *II*, *IV*, *IX*, and *XII*, we prepared ethyl esters of CEO derivatives *XVI*, *XIX*, *XXIX*, and *XXXV*, respectively in yields of 59 – 83%. In the case of oxime *II* the addition of methyl acrylate was also checked, but the yield of *XV* was only 45%. Acetyl derivatives *IV*, *IX*, and *XII* are simultaneously cleaved, yielding corresponding 3-hydroxy derivatives, whereas the adduct from the methoxymethyl derivative *II* can be deprotected in the separate step in acid medium. The completion of the whole set of CEO derivatives

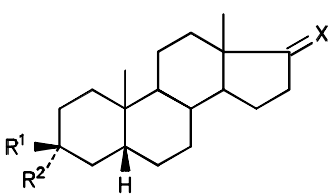


	R	X
I	MOM	O
II	MOM	N–OH (17*E*)
III	Ac	O
IV	Ac	N–OH (17*E*)
V	Ac	N–OH (17*Z*)
VI	H	O

MOM = methoxymethyl



	R^1	R^2	X
VII	H	OH	O
VIII	OAc	H	O
IX	OAc	H	N–OH (17*E*)
X	OH	H	O



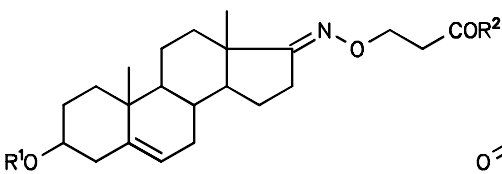
	R^1	R^2	X
XI	H	OAc	O
XII	H	OAc	N–OH (17*E*)
XIII	H	OH	O
XIV	OH	H	O

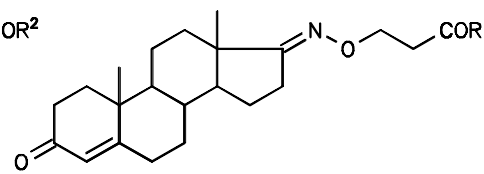
differing in positions 3 and 5 was accomplished by the reaction of (*O*-(2-carboxyethyl))hydroxylamine with ketones *I*, *III*, *VI*, *VII*, *X*, *XIII*, and *XIV*, giving corresponding CEO derivatives *XVII*, *XVIII*, *XX*, *XXVII*, *XXX*, *XXXVI*, and *XXXVIII*.

The ester group hydrolysis by 2 M aqueous sodium hydroxide in both the CEO methyl ester *XV* and CEO ethyl ester *XVI* gave free acid, i.e. CEO derivative *XVII*. Analogical hydrolysis of ethyl esters *XIX*, *XXIX*, and *XXXV* afforded CEO derivatives *XX*, *XXX*, and *XXXVI*, respectively.

By the Oppenauer oxidation[5] of 3β-hydroxy-5-ene derivative *XIX* the 3-oxo-4-ene derivative *XXIV* was prepared and 3-hydroxy derivatives *XXIX* and *XXXV* were oxidized by Jones reagent into the corresponding 3-keto derivatives *XXXII* and *XL*. So obtained esters were hydrolyzed into the CEO derivatives *XXV*, *XXXIII*, and *XLI*.

The set of CEO derivatives (*XVII*, *XVIII*, *XX*, *XXV*, *XXVII*, *XXX*, *XXXIII*, *XXXVI*, *XXXVIII*, and *XLI*) was transformed by the modification of the known procedure[6] into

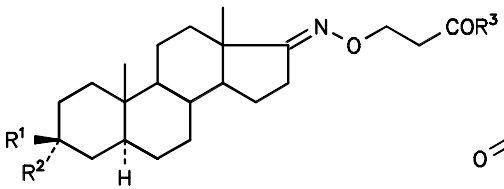




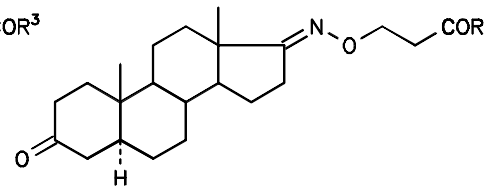
	R^1	R^2
XV	MOM	OCH_3
XVI	MOM	OC_2H_5
XVII	MOM	OH
XVIII	Ac	OH
XIX	H	OC_2H_5
XX	H	OH
XXI	MOM	OSu
XXII	Ac	OSu
XXIII	H	OSu

XXIV, R = OC_2H_5
XXV, R = OH
XXVI, R = OSu

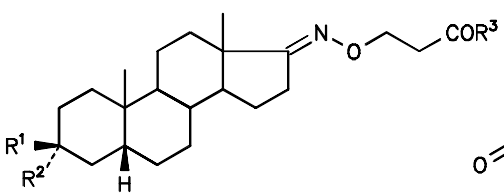
Su = *N*-succinimidyl
MOM = methoxymethyl



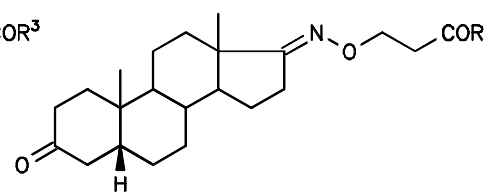
	R^1	R^2	R^3
XXVII	H	OH	OH
XXVIII	H	OH	OSu
XXIX	OH	H	OC_2H_5
XXX	OH	H	OH
XXXI	OH	H	OSu



XXXII, R = OC_2H_5
XXXIII, R = OH
XXXIV, R = OSu



	R^1	R^2	R^3
XXXV	H	OH	OC_2H_5
XXXVI	H	OH	OH
XXXVII	H	OH	OSu
XXXVIII	OH	H	OH
XXXIX	OH	H	OSu



XL, R = OC_2H_5
XLI, R = OH
XLII, R = OSu

Su = *N*-succinimidyl

the corresponding active esters with *N*-hydroxysuccinimide (*XXI* – *XXIII*, *XXVI*, *XXVIII*, *XXXI*, *XXXIV*, *XXXVII*, *XXXIX*, and *XLII*). Their structure was confirmed by the presence of characteristic bands in the IR spectra (ca 1 820, 1 780, and 1 740 cm^{-1}) and by the signal at δ 2.83 ppm in the ^1H NMR spectra.

Generally, ^1H NMR spectra of 17-CEO derivatives under study exhibit characteristic signals belonging to OCH_2CH_2COOR moiety. Its four protons are arranged in two triplets with the coupling constant about 6 Hz, first at δ ca 4.3 ppm (OCH_2), second at δ 2.63 – 2.97 ppm (CH_2COO). In the mass spectra of ethyl esters of CEO derivatives in addition to the molecular ions the characteristic ions resulted by splitting of $OCH_2CH_2COOCH_2CH_3$ (*m*/*z* 117) fragment are formed.

The configuration at the double bond C=N of 17-CEO derivatives was studied by ^1H and ^{13}C NMR methods. In the ^1H NMR spectra of isomeric 17-oximino derivatives *IV* and *V* the singlets of H-18 angular methyl protons are found for (17*E*)-isomer *IV* at δ 0.93 ppm and for (17*Z*)-isomer *V* at δ 1.05 ppm (see ref.[7]). In analogy, comparing the corresponding chemical shifts of H-18 (Table I), the (17*E*)-configuration was assigned for the CEO-derivatives under study. ^{13}C NMR spectra of compounds *XIX*, *XXIX*, and *XXXV* (Table II) display the chemical shifts of carbons in the proximity of C=N bond similar to corresponding set in (17*E*)-isomer *IV*, differing from the data for (17*Z*)-isomer *V*.

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^1H NMR spectra were taken on a Varian UNITY-200 (200 MHz, FT mode) and ^{13}C NMR spectra on a Varian UNITY-500 (125.7 MHz, FT mode) spectrometer at 23 °C in deuteriochloroform with tetramethylsilane as internal standard unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) in Hz. For ^{13}C NMR spectra the number of directly bonded hydrogen atoms was determined from the proton decoupled "attached proton test" (ref.[8]). FAB mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography was performed on silica gel G (ICN Biochemicals), with detection by spraying with concentrated sulfuric acid followed by heating. For column chromatography silica gel 60 – 120 μm was used. Prior to evaporation on rotary evaporator in vacuo (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

General Procedure for Preparation of Oximes *II*, *IX*, and *XII*

Hydroxylamine hydrochloride (3.47 g, 50 mmol) was added to a solution of ketone (15 mmol) in pyridine (50 ml). After stirring at 60 °C for 4 h, the solvent was evaporated in vacuo and the residue was partitioned between ether and water. The aqueous phase was extracted with ether and combined organic phases were washed successively with dilute hydrochloric acid (1 : 4, 3 times), water, saturated potassium hydrogen carbonate solution and water. After drying the solvent was evaporated in vacuo. The residue was chromatographed on silica gel column (250 g) in benzene–ether (90 : 10) (oxime *II*) or crystallized from methanol–water (oximes *IX* and *XII*).

TABLE I

^{1}H NMR spectral parameters of (17*E*)-17-oximinoandrostane derivatives. Measured in $CDCl_3$, for other conditions see Experimental

Compound	18-H_3 s	19-H_3 s	3-H m (*W*)	6-H bd (*J*)	OCH_2 t (*J*)	CH_2COO t (*J*)
			5-ene derivatives			
XV[a,b]	0.91	1.03	[c]	5.36 (4)	4.28 (6.0)	2.65 (6.0)
XVI[b,d]	0.91	1.03	[c]	5.36 (4)	4.28 (6.0)	2.67 (6.0)
XVII[b]	0.92	1.03	3.43 (33)	5.36 (4.5)	4.29 (6.0)	2.73 (6.0)
XVIII[e]	0.92	1.04	4.60 (34)	5.38 (4)	4.29 (6.0)	2.74 (6.0)
XIX[d]	0.91	1.03	3.52 (34)	5.35 (5)	4.28 (6.5)	2.63 (6.5)
XX[f]	0.85	0.97	[c]	5.28 (4)	4.11 (6.5)	2.49 (6.5)
XXI[b,g]	0.92	1.03	[c]	5.36 (4.5)	4.36 (6.0)	2.96 (6.0)
XXII[e,g]	0.91	1.03	4.56 (32)	5.38 (4.5)	4.35 (6.2)	2.96 (6.2)
XXIII[g]	0.91	1.02	3.52 (33)	5.35 (4.5)	4.35 (6.4)	2.96 (6.4)
			4-ene derivatives			
XXIV[d,h]	0.94	1.20	–	[c]	4.27 (6.5)	2.63 (6.5)
XXV[h]	0.95	1.20	–	[c]	4.29 (6.5)	2.72 (6.5)
XXVI[g,h]	0.95	1.21	–	[c]	4.36 (6.2)	2.96 (6.2)
			5α-derivatives			
XXVII	0.89	0.79	4.05 (11)	[c]	4.28 (5.9)	2.74 (5.9)
XXVIII[g]	0.89	0.80	4.05 (12)	[c]	4.36 (6.3)	2.97 (6.3)
XXIX[d]	0.88	0.82	3.59 (32)	[c]	4.27 (6.5)	2.63 (6.5)
XXX	0.89	0.82	3.61 (32)	[c]	4.28 (6.0)	2.73 (6.0)
XXXI[g]	0.89	0.83	3.59 (31)	[c]	4.35 (6.2)	2.96 (6.2)
XXXII[d]	0.91	1.03	–	[c]	4.28 (6.5)	2.63 (6.5)
XXXIII	0.93	1.03	–	[c]	4.29 (5.9)	2.75 (5.9)
XXXIV[g]	0.92	1.03	–	[c]	4.36 (6.0)	2.96 (6.0)
			5β-derivatives			
XXXV[d]	0.87	0.94	3.63 (31)	[c]	4.28 (6.5)	2.63 (6.5)
XXXVI	0.89	0.94	3.59 (32)	[c]	4.29 (5.9)	2.74 (5.9)
XXXVII[g]	0.87	0.93	3.62 (32)	[c]	4.35 (6.1)	2.96 (6.1)
XXXVIII	0.89	0.98	4.12 (12)	[c]	4.28 (5.8)	2.73 (5.8)
XXXIX[g]	0.89	0.99	4.12 (14)	[c]	4.36 (6.3)	2.97 (6.3)
XL[d]	0.91	1.04	–	[c]	4.28 (6.5)	2.63 (6.5)
XLI	0.92	1.04	–	[c]	4.28 (6.0)	2.73 (6.0)
XLII[g]	0.92	1.05	–	[c]	4.36 (6.3)	2.97 (6.3)

[a] Other signal 3.69 s ($COOCH_3$). [b] Other signals 3.37 s and 4.69 s (OCH_2OCH_3). [c] Undeterminable value. [d] Other signals 1.26 t and 4.15 q (OCH_2CH_3, J = 7.2). [e] Other signal 2.03 s ($OOCCH_3$). [f] Measured in CD_3SOCD_3. [g] Other signal 2.83 s (4 H, *N*-hydroxysuccinimide residue). [h] Other signal 5.74 bs (4-H).

(17E)-3β-Methoxymethoxyandrost-5-en-17-one oxime (II). Ketone[9] *I* (4.99 g, 15 mmol) afforded 4.34 g (83%) of oxime *II*, m.p. 180 – 182 °C, $[\alpha]_D$ −61° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 587, 3 298 (O–H); 1 669 (C=N); 1 148, 1 102, 1 036, 911 (OCH_2OCH_3). ^{1}H NMR spectrum: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.37 s, 3 H (OCH_3); 4.69 s, 2 H (OCH_2O); 5.37 bd, 1 H (H-6, J = 4.5); 8.24 bs, 1 H (C=N–OH). For $C_{21}H_{33}NO_3$ (347.5) calculated: 72.58% C, 9.57% H, 4.03% N; found: 72.37% C, 9.33% H, 3.88% N.

(17E)-3β-Acetoxy-5α-androstan-17-one oxime (IX). Ketone *VIII* (4.99 g, 15 mmol) afforded 4.92 g (94%) of oxime *IX*, m.p. 187 – 189 °C, $[\alpha]_D$ +8° (*c* 0.4, chloroform). Literature[10] gives m.p. 182 °C, $[\alpha]_D$ +5° (*c* 1.015, chloroform). IR spectrum (chloroform): 3 584, 3 283 (O–H); 1 723 (C=O); 1 261 1 026 (C–O). ^{1}H NMR spectrum: 0.84 s, 3 H (3 × H-19); 0.90 s, 3 H (3 × H-18); 2.02 s, 3 H (CH_3COO); 4.69 m, 1 H (H-3α, W = 32); 8.46 bs, 1 H (C=N–OH).

TABLE II
^{13}C NMR spectral parameters of 17-oximinoandrostane derivatives. Measured in $CDCl_3$, for other conditions see Experimental

Carbon	*XIX*	*XXIX*	*XXXV*	*IV*[a]	*V*[a]
1	37.13	36.86	36.27	36.9	37.0
2	31.46[b]	28.40	30.41	27.2	27.8
3	71.43	70.95	71.56	73.6	73.9
4	42.11	34.04	35.27[c]	38.0	38.2
5	140.96	44.72	41.96	139.2	140.1
6	120.95	31.31[d]	26.92	121.7	122.1
7	31.27[b]	31.45[d]	25.96[e]	31.2	31.4
8	31.19	34.77	35.18	31.2	31.4
9	50.18	53.73[f]	40.67	50.2	50.4
10	36.52	35.50	34.65[c]	36.6	35.2
11	20.49	20.66	20.26	20.4	20.6
12	34.87[g]	37.97	34.24	33.9	34.1
13	43.72	43.96	44.07	43.6	45.8
14	54.02	54.39[f]	53.82	54.0	54.2
15	23.19	23.04	23.12	23.3	23.3
16	25.67	25.60	25.71[e]	25.0	29.1
17	170.99[h]	171.18[i]	171.21[j]	170.2	171.2
18	16.90	17.13	17.07	16.9	13.7
19	19.33	12.20	23.22	19.3	19.4
O**C**H_2CH_2COO	68.54	68.48	68.53		
OCH_2**C**H_2COO	33.92[g]	34.86	34.89		
COO	171.63[h]	171.61[i]	171.65[j]		
COO**C**H_2CH_3	60.32	60.28	60.31		
$COOCH_2$**C**H_3	14.15	14.13	14.15		

[a] Values taken from ref.[7]. [b – j] Signals can be mutually interchanged.

(17E)-3α-Acetoxy-5β-androstan-17-one oxime (XII). Ketone *XI* (4.99 g, 15 mmol) afforded 3.44 g (66%) of oxime *XII*, m.p. 187 – 189 °C, $[\alpha]_D$ +54° (*c* 1.8, chloroform). IR spectrum (chloroform): 3 588, 3 288 (O–H); 1 720 (C=O); 1 253, 1 028 (C–O). ^{1}H NMR spectrum: 0.90 s, 3 H (3 × H-18); 0.96 s, 3 H (3 × H-19); 2.03 s, 3 H (CH_3COO); 4.73 m, 1 H (H-3β, *W* = 32); 8.18 bs, 1 H (C=N–OH). For $C_{21}H_{33}NO_3$ (347.5) calculated: 72.58% C, 9.57% H, 4.03% N; found: 72.37% C, 9.33% H, 3.88% N.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one 17-(*O*-(2-Carboxyethyl))oxime Methyl Ester (*XV*)

Methyl acrylate (0.70 ml, 7.8 mmol) was added to the mixture of oxime *II* (695 mg, 2 mmol), benzene (4 ml), methanol (4 ml) and 2 M solution of potassium hydroxide in methanol (0.4 ml). After stirring at 50 °C for 5 h, the solvents were evaporated in vacuo, and the residue was partitioned between ether and water. The water phase was extracted with ether and combined organic phases were washed with water (3 times), dried and evaporated in vacuo. The residue was chromatographed on silica gel column (40 g), mixture benzene–ether (95 : 5) eluted 390 mg (45%) of ester *XV*, m.p. 62 – 64 °C (methanol), $[\alpha]_D$ −80° (*c* 0.2, chloroform). IR spectrum (tetrachloromethane): 1 745 (C=O); 1 175 (C–O, ester); 1 150, 1 106, 1 047, 1 039, 918 (C–O). Mass spectrum, *m*/*z*: 433 (M^+), 418, 371 (M – 62), 268 (371 – $OCH_2CH_2COOCH_3$). For $C_{25}H_{39}NO_5$ (433.6) calculated: 69.25% C, 9.07% H, 3.23% N; found: 69.45% C, 8.75% H, 3.01% N.

General Procedure for Reaction of Oximes *II*, *IV*, *IX*, and *XII* with Ethyl Acrylate

Ethyl acrylate (4.33 ml, 40 mmol) was added to the mixture of oxime (10 mmol), tetrahydrofuran (15 ml), ethanol (15 ml) and 2 M solution of potassium hydroxide in ethanol (4.0 ml). After stirring at 50 °C for 5 h, another portions of ethyl acrylate (4.33 ml, 40 mmol) and 2 M solution of potassium hydroxide in ethanol (4.0 ml) was added. The mixture was stirred at 50 °C for another 5 h, the solvents were evaporated in vacuo, and the residue was partitioned between ether and water. The water phase was extracted with ether and combined organic phases were washed with water (3 times), dried and evaporated in vacuo. The residue was chromatographed on silica gel column (300 g), in benzene–ether (90 : 10, for compounds *XIX* and *XXIX*) or in benzene–ether (95 : 5, for compounds *XVI* and *XXXV*)

(17E)-3β-Methoxymethoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime ethyl ester (XVI). Oxime *II* (3.48 g) gave 3.73 g (83%) of oily oxime *XVI*, $[\alpha]_D$ −31° (*c* 0.4, chloroform). IR spectrum (tetrachloromethane): 1 739 (C=O); 1 183 (C–O, ester); 1 149, 1 106, 1 039, 918 (C–O, OCH_2OCH_3). Mass spectrum, *m*/*z*: 447 (M^+), 385 (M – 62), 330 (M – $OCH_2CH_2COOCH_2CH_3$), 268 (385 – $OCH_2CH_2COOCH_2CH_3$). For $C_{26}H_{41}NO_5$ (447.6) calculated: 69.77% C, 9.23% H, 3.13% N; found: 70.03% C, 8.98% H, 2.97% N.

(17E)-3β-Hydroxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime ethyl ester (XIX). Oxime[7] *IV* (3.46 g) afforded 2.73 g (68%) of oxime *XIX*, m.p. 74 – 76 °C (light petroleum), $[\alpha]_D$ −43° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 609 (O–H); 1 729 (C=O); 1 186, 1 086 (C–O). Mass spectrum, *m*/*z*: 403 (M^+), 286 (M – $OCH_2CH_2COOCH_2CH_3$), 271 (286 – CH_3), 268 (286 – H_2O). For $C_{24}H_{37}NO_4$ (403.6) calculated: 71.43% C, 9.24% H, 3.47% N; found: 71.25% C, 9.00% H, 3.76% N.

(17E)-3β-Hydroxy-5α-androstan-17-one 17-(O-(2-carboxyethyl))oxime ethyl ester (XXIX). Oxime *IX* (3.48 g) afforded 2.38 g (59%) of oxime *XXIX*, m.p. 71 – 73 °C (light petroleum), $[\alpha]_D$ +28° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 608, 3 446 (O–H); 1 726 (C=O); 1 653 (C=N); 1 188, 1 036 (C–O). Mass spectrum, *m*/*z*: 405 (M^+), 288 (M – $OCH_2CH_2COOCH_2CH_3$), 273 (286 – CH_3). For $C_{24}H_{39}NO_4$ (405.6) calculated: 71.07% C, 9.69% H, 3.45% N; found: 69.88% C, 9.75% H, 3.23% N.

(17E)-3α-Hydroxy-5β-androstan-17-one 17-(O-(2-carboxyethyl))oxime ethyl ester (XXXV). Oxime *XII* (3.48 g) afforded 2.56 g (63%) of oily oxime *XXXV*, $[\alpha]_D$ +39° (*c* 0.5, chloroform). IR spectrum (chloroform): 3 609, 3 443 (O–H); 1 729 (C=O); 1 188 (C–O). Mass spectrum, *m/z*: 405 (M^+), 288 (M – $OCH_2CH_2COOCH_2CH_3$), 273 (286 – CH_3), 270 (288 – H_2O), 255 (270 – CH_3). For $C_{24}H_{39}NO_4$ (405.6) calculated: 71.07% C, 9.69% H, 3.45% N; found: 69.92% C, 9.89% H, 3.37% N.

(17*E*)-3β-Hydroxyandrost-5-en-17-one 17-(*O*-(2-Carboxyethyl))oxime Ethyl Ester (*XIX*)

p-Toluenesulfonic acid monohydrate (875 mg, 4.6 mmol) was added to a solution of methoxymethoxy derivative *XVI* (895 mg, 2 mmol) in a mixture of benzene (15 ml) and ethanol (30 ml). The mixture was warmed to 45 °C for 7 h, the solvents were evaporated in vacuo, the residue was partitioned between ether and water and the aqueous phase was extracted with ether. The organic phases were combined and washed successively with water, saturated aqueous potassium hydrogen carbonate solution and water. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (50 g). Benzene–ether (90 : 10) washed out 548 mg (68%) of the hydroxy derivative *XIX*, m.p. 74 – 76 °C (light petroleum), identical with product prepared by reaction of oxime *IV* with ethyl acrylate.

(17*E*)-Androst-4-ene-3,17-dione 17-(*O*-(2-Carboxyethyl))oxime Ethyl Ester (*XXIV*)

1-Methyl-4-piperidone (2.0 ml, 16.3 mmol) was added under argon to a solution of hydroxy derivative *XIX* (1.21 g, 3.0 mmol) in toluene (40 ml). A part (5 ml) of toluene was distilled off and a solution of aluminum isopropoxide (613 mg, 3.0 mmol) in toluene (5 ml) was added. After refluxing under argon for 2 h, the mixture was cooled, diluted with ether (200 ml) and successively washed with dilute hydrochloric acid (1 : 4), water, saturated aqueous solution of potassium hydrogen carbonate and with water. The solvents was taken down, the residue was chromatographed on column of silica gel (100 g) in benzene–ether (95 : 5) to afford 964 mg (80%) of oily ketone *XXIV*, $[\alpha]_D$ +112° (*c* 0.7, chloroform). IR spectrum (tetrachloromethane): 1 738 (C=O, ester); 1 679 (C=O, ketone); 1 619 (C=C); 1 184, 1 040 (C–O). Mass spectrum, *m/z*: 401 (M^+), 284 (M – $OCH_2CH_2COOCH_2CH_3$), 269 (284 – CH_3). For $C_{24}H_{35}NO_4$ (401.6) calculated: 71.79% C, 8.79% H, 3.49% N; found: 71.95% C, 8,65% H, 3.32% N.

(17*E*)-5α-Androstane-3,17-dione 17-(*O*-(2-Carboxyethyl))oxime Ethyl Ester (*XXXII*)

Jones reagent (0.8 ml) was added to a solution of hydroxy derivative *XXIX* (730 mg, 1.8 mmol) in acetone (15 ml). After stirring for 10 min at room temperature the excess reagent was decomposed by methanol (5 ml). The solvents were evaporated in vacuo, the residue was partitioned between ether and water. Water phase was extracted with ether, the combined organic phases was washed with water, saturated aqueous solution of potassium hydrogen carbonate and water. The solvent was taken down, the residue was chromatographed on column of silica gel (40 g) in benzene–ether (90 : 10) to afford 713 mg (98%) of oily ketone *XXXII*, $[\alpha]_D$ +51° (*c* 0.7, chloroform). IR spectrum (tetrachloromethane): 1 739 (C=O, ester); 1 716 (C=O, ketone); 1 183 (C–O). Mass spectrum, *m/z*: 403 (M^+), 388 (M – CH_3), 358 (M – OCH_2CH_3), 286 (M – $OCH_2CH_2COOCH_2CH_3$), 271 (286 – CH_3). For $C_{24}H_{37}NO_4$ (403.6) calculated: 71.43% C, 9.24% H, 3.47% N; found: 71.25% C, 9.00% H, 3.76% N.

(17*E*)-5β-Androstane-3,17-dione 17-(*O*-(2-Carboxyethyl))oxime Ethyl Ester (*XL*)

The title compound was prepared from hydroxy derivative *XXXV* (730 mg, 1.8 mmol) as described for the preparation of ketone *XXXII* from hydroxy derivative *XXIX* (see preceding experiment). Yield 713 mg (98%) of oily ketone *XL*, $[\alpha]_D$ +43° (*c* 0.3, chloroform). IR spectrum (tetrachloromethane): 1 739 (C=O, ester); 1 716 (C=O, ketone); 1 183 (C–O). Mass spectrum, *m/z*: 403 (M^+), 358 (M – OCH_2CH_3), 286 (M – $OCH_2CH_2COOCH_2CH_3$), 271 (286 – CH_3). For $C_{24}H_{37}NO_4$ (403.6) calculated: 71.43% C, 9.24% H, 3.47% N; found: 71.22% C, 8.95% H, 3.78% N.

General Procedure for Reaction of 17-Oxo Derivatives *I*, *III*, *VI*, *VII*, *X*, *XIII*, and *XIV* with (*O*-(2-Carboxyethyl))hydroxylamine Hydrochloride

(*O*-(2-Carboxyethyl))hydroxylamine hydrochloride[1] (283 mg, 2.0 mmol) was added to a solution of 17-oxo derivative (1.0 mmol) in pyridine (3.5 ml). After stirring at 60 °C for 6 h, the solvent was evaporated in vacuo, the residue was coevaporated with toluene (60 ml) and partitioned between ether and dilute hydrochloric acid (1 : 4). Aqueous phase was extracted with ether and combined organic phases was washed successively with dilute hydrochloric acid (1 : 4, 3 times) and water (3 times). After drying the solvent was evaporated in vacuo. The residue was crystallized from light petroleum–ether, unless stated otherwise.

*(17E)-3*β*-Methoxymethoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime* (XVII). Ketone[9] *I* (333 mg, 1 mmol) afforded 342 mg (81%) of product *XVII*, m.p. 120 – 122 °C, $[\alpha]_D$ –20° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 714 (C=O); 1 148, 1 103, 1 037, 911 (OCH_2CH_3). For $C_{24}H_{37}NO_5$ (419.6) calculated: 68.71% C, 8.89% H, 3.34% N; found: 68.75% C, 8.74% H, 3.11% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime* (XVIII). Ketone *III* (331 mg, 1 mmol) gave 293 mg (70%) of product *XVIII*, m.p. 141 – 143 °C, $[\alpha]_D$ –38° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 714 (C=O); 1 669 (C=C); 1 255 (C–O, acetate); 1 034 (C–O). For $C_{24}H_{35}NO_5$ (417.6) calculated: 69.04% C, 8.45% H, 3.35% N; found: 68.74% C, 8.73% H, 3.55% N.

*(17E)-3*β*-Hydroxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime* (XX). Ketone *VI* (289 mg, 1 mmol) afforded after crystallization from ethyl acetate–light petroleum 260 mg (69%) of product *XX*, m.p. 177 – 180 °C, $[\alpha]_D$ –43° (*c* 0.5, chloroform). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 422 (O–H); 1 702 (C=O); 1 044 (C–O). For $C_{22}H_{33}NO_4$ (375.5) calculated: 70.37% C, 8.86% H, 3.73% N; found: 70.38% C, 8.83% H, 3.53% N.

*(17E)-3*α*-Hydroxy-5*α*-androstan-17-one 17-(O-(2-carboxyethyl))oxime* (XXVII). Ketone *VII* (291 mg, 1 mmol) gave 277 mg (73%) of product *XXVII*, m.p. 188 – 191 °C, $[\alpha]_D$ +37° (*c* 0.4, chloroform). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 536 (O–H); 1 725 (C=O). For $C_{22}H_{35}NO_4$ (377.5) calculated: 69.99% C, 9.34% H, 3.71% N; found: 69.85% C, 9.33% H, 3.62% N.

*(17E)-3*β*-Hydroxy-5*α*-androstan-17-one 17-(O-(2-carboxyethyl))oxime* (XXX). Ketone *X* (291 mg, 1 mmol) afforded 280 mg (74%) of product *XXX*, m.p. 179 – 181 °C, $[\alpha]_D$ +34° (*c* 0.5, chloroform). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 434, 3 248 (O–H); 1 705 (C=O); 1 041 (C–O). For $C_{22}H_{35}NO_4$ (377.5) calculated: 69.99% C, 9.34% H, 3.71% N; found: 69.79% C, 9.27% H, 3.72% N.

*(17E)-3*α*-Hydroxy-5*β*-androstan-17-one 17-(O-(2-carboxyethyl))oxime* (XXXVI). Ketone *XIII* (291 mg, 1 mmol) gave 310 mg (82%) of product *XXXVI*, m.p. 142 – 144 °C, $[\alpha]_D$ +44° (*c* 0.4, chloroform). IR spectrum (chloroform): 3 607 (O–H); 3 500 – 2 500 (COOH); 1 714 (C=O). For $C_{22}H_{35}NO_4$ (377.5) calculated: 69.99% C, 9.34% H, 3.71% N; found: 70.09% C, 9.52% H, 3.35% N.

(17E)-3β-Hydroxy-5β-androstan-17-one 17-(O-(2-carboxyethyl))oxime (XXXVIII). Ketone *XIV* (291 mg, 1 mmol) afforded 350 mg (93%) of crude amorphous product *XXXVIII*, which was used without purification. IR spectrum (chloroform): 3 615 (O–H); 3 500 – 2 500 (COOH); 1 714 (C=O); 1 035 (C–O).

General Procedure for Hydrolysis of (*O*-(2-Carboxyethyl)oxime Esters *XV*, *XVI*, *XIX*, *XXIV*, *XXIX*, *XXXII*, *XXXV*, and *XL*

Ester (0.5 mmol) in methanol (10 ml) was at room temperature treated under stirring with a 2 M aqueous potassium hydroxide (5 ml). After 12 h the reaction mixture was neutralized with dilute hydrochloric acid (1 : 4), the solvents were taken down in vacuo and the residue was partitioned between ether and water. Aqueous phase was extracted with ether, the combined organic phases were washed with water. The solvents was taken down, the residue was crystallized from light petroleum–ether, unless stated otherwise.

(17E)-3β-Methoxymethoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime (XVII). Method *A*. Methyl ester *XV* (217 mg, 0.5 mmol) give 131 mg (62%) of acid *XVII*, m.p. 119 – 121 °C, identical with a sample prepared from ketone *I* and (*O*-(2-carboxyethyl))hydroxylamine hydrochloride. Method *B*. Ethyl ester *XVI* (224 mg, 0.5 mmol) afforded 165 mg (79%) of acid *XVII*, m.p. 119 – 122 °C, identical with the product obtained by procedure *A*.

(17E)-3β-Hydroxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime (XX). Ethyl ester *XIX* (202 mg, 0.5 mmol) afforded after crystallization from light petroleum–ethyl acetate 130 mg (69%) of acid *XX*, m.p. 179 – 182 °C, identical with sample prepared from ketone *VI* and (*O*-(2-carboxyethyl))hydroxylamine hydrochloride.

(17E)-Androst-4-ene-3,17-dione 17-(O-(2-carboxyethyl))oxime (XXV). Ethyl ester *XXIV* (201 mg, 0.5 mmol) give 312 mg (83%) of acid *XXV*, m.p. 184 – 187 °C, $[\alpha]_D$ +111° (*c* 0.5, chloroform). IR spectrum (chloroform): 3 500 – 2 500, 1 714 (COOH); 1 664 (C=O); 1 616 (C=C). For $C_{22}H_{31}NO_4$ (373.5) calculated: 70.75% C, 8.37% H, 3.75% N; found: 70.87% C, 8.62% H, 3.47% N.

(17E)-3β-Hydroxy-5α-androstan-17-one 17-(O-(2-carboxyethyl))oxime (XXX). Ethyl ester *XXIX* (203 mg, 0.5 mmol) give 137 mg (73%) of acid *XXX*, m.p. 177 – 180 °C (light petroleum–ether), identical with sample prepared from ketone *X* and (*O*-(2-carboxyethyl))hydroxylamine hydrochloride.

(17E)-5α-Androstane-3,17-dione 17-(O-(2-carboxyethyl))oxime (XXXIII). Ethyl ester *XXXII* (202 mg, 0.5 mmol) afforded 108 mg (57%) of acid *XXXIII*, m.p. 102 – 104 °C, $[\alpha]_D$ +60° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 710 (C=O). For $C_{22}H_{33}NO_4$ (375.5) calculated: 70.37% C, 8.86% H, 3.73% N; found: 70.38% C, 8.83% H, 3.53% N.

(17E)-3α-Hydroxy-5β-androstan-17-one 17-(O-(2-carboxyethyl))oxime (XXXVI). Ethyl ester *XXXV* (203 mg, 0.5 mmol) afforded 125 mg (66%) of acid *XXXVI*, m.p. 140 – 143 °C (light petroleum–ether), identical with a sample prepared from ketone *XIII* and (*O*-(2-carboxyethyl))hydroxylamine hydrochloride.

(17E)-5β-Androstane-3,17-dione 17-(O-(2-carboxyethyl))oxime (XLI). Ethyl ester *XL* (202 mg, 0.5 mmol) afforded 184 mg (98%) of amorphous acid *XLI*, which was used without purification. IR spectrum (chloroform): 3 500 – 2 500 (COOH); 1 710 (C=O).

General Procedure for Preparation of *N*-Succinimidyl Esters *XXI – XXIII*, *XXVI*, *XXVIII*, *XXXI*, *XXXIV*, *XXXVII*, *XXXIX*, and *XLII*

To a mixture of acid (0.2 mmol), *N*-hydroxysuccimide (35 mg, 0.3 mmol), 4-dimethylaminopyridine (2.5 mg, 20 μmol) and tetrahydrofuran (1.5 ml) was added 1 M solution of *N*,*N*′-dicyclohexylcarbodiimide in benzene (0.3 ml). After stirring at room temperature for 12 h, the precipitated *N*,*N*′-dicyclohexylurea was filtered off and the solvent was taken down. The residue was chromato-

graphed on two preparative silica gel plates in benzene–ether (8 : 2, for compounds *XXI*, *XXII*, *XXVI*, *XXXIV*, and *XLII*) or in benzene–ethyl acetate (1 : 1, for compounds *XXIII*, *XXVIII*, *XXXI*, *XXXVII*, and *XXXIX*).

(17E)-3β-Methoxymethoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXI). The acid *XVII* (84 mg) afforded 82 mg (79%) of ester *XXI*, m.p. 168 – 171 °C (ether), $[\alpha]_D$ −32° (*c* 0.3, chloroform). IR spectrum (chloroform): 1 819, 1 788 (C=O, imide); 1 742 (C=O, ester); 1 148, 1 102, 1 037, 910 (OCH_2OCH_3). For $C_{28}H_{40}N_2O_7$ (516.6) calculated: 65.10% C, 7.80% H, 5.42% N; found: 65.35% C, 8.03% H, 5.67% N.

(17E)-3β-Acetoxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXII). The acid *XVIII* (84 mg) afforded 95 mg (92%) of ester *XXII*, m.p. 126 – 128 °C (ether), $[\alpha]_D$ −37° (*c* 0.3, chloroform). IR spectrum (chloroform): 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester); 1 255, 1 043 (C–O). For $C_{28}H_{38}N_2O_7$ (514.6) calculated: 65.35% C, 7.44% H, 5.44% N; found: 65.16% C, 7.13% H, 5.73% N.

(17E)-3β-Hydroxyandrost-5-en-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXIII). The acid *XX* (75 mg) afforded 87 mg (92%) of ester *XXIII*, m.p. 171 – 174 °C (methanol), $[\alpha]_D$ −42° (*c* 0.2, chloroform). IR spectrum (chloroform): 3 608 (O–H); 1 819, 1 788 (C=O, imide); 1 741 (C=O, ester). For $C_{26}H_{36}N_2O_6$ (472.6) calculated: 66.08% C, 7.68% H, 5.93% N; found: 66.35% C, 7.93% H, 6.21% N.

(17E)-Androst-4-ene-3,17-dione 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXVI). The acid *XXV* (75 mg) afforded 75 mg (79%) of ester *XXVI*, m.p. 156 – 159 °C (ether), $[\alpha]_D$ +77° (*c* 0.3, chloroform). IR spectrum (chloroform): 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester); 1 664 (C=O, ketone); 1 615 (C=C). For $C_{26}H_{34}N_2O_6$ (470.6) calculated: 66.36% C, 7.28% H, 5.95% N; found: 66.55% C, 6.97% H, 6.07% N.

(17E)-3α-Hydroxy-5α-androstan-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXVIII). The acid *XXVII* (75 mg) afforded 89 mg (94%) of ester *XXVIII*, m.p. 173 – 175 °C (ether), $[\alpha]_D$ +22° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 616 (O–H); 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester). For $C_{26}H_{38}N_2O_6$ (474.6) calculated: 65.80% C, 8.07% H, 5.90% N; found: 65.65% C, 8.23% H, 5.74% N.

(17E)-3β-Hydroxy-5α-androstan-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXXI). The acid *XXX* (75 mg) afforded 90 mg (95%) of ester *XXXI*, m.p. 178 – 181 °C (ether), $[\alpha]_D$ +19° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 609 (O–H); 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester). For $C_{26}H_{38}N_2O_6$ (474.6) calculated: 65.80% C, 8.07% H, 5.90% N; found: 65.63% C, 7.94% H, 6.18% N.

(17E)-5α-Androstane-3,17-dione 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXXIV). The acid *XXXIII* (75 mg) afforded 71 mg (75%) of ester *XXXIV*, m.p. 191 – 194 °C (ether), $[\alpha]_D$ +38° (*c* 0.3, chloroform). IR spectrum (chloroform): 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester); 1 707 (C=O, ketone). For $C_{26}H_{36}N_2O_6$ (472.6) calculated: 66.08% C, 7.68% H, 5.93% N; found: 65.87% C, 7.76% H, 6.09% N.

(17E)-3α-Hydroxy-5β-androstan-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXXVII). The acid *XXXVI* (75 mg) afforded 88 mg (93%) of ester *XXXVII*, m.p. 119 – 122 °C (ether), $[\alpha]_D$ +23° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 606 (O–H); 1 819, 1 788 (C=O, imide); 1 741 (C=O, ester). For $C_{26}H_{38}N_2O_6$ (474.6) calculated: 65.80% C, 8.07% H, 5.90% N; found: 66.07% C, 7.81% H, 6.04% N.

(17E)-3β-Hydroxy-5β-androstan-17-one 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XXXIX). The acid *XXXVIII* (75 mg) afforded 69 mg (73%) of ester *XXXIX*, m.p. 149 – 152 °C (ether), $[\alpha]_D$ +20° (*c* 0.3, chloroform). IR spectrum (chloroform): 3 615 (O–H); 1 819, 1 788 (C=O, imide); 1 742 (C=O, ester). For $C_{26}H_{38}N_2O_6$ (474.6) calculated: 65.80% C, 8.07% H, 5.90% N; found: 65.72% C, 7.88% H, 6.14% N.

(17E)-5β-Androstane-3,17-dione 17-(O-(2-carboxyethyl))oxime N-succinimidyl ester (XLII). The acid *XLI* (75 mg) afforded 83 mg (88%) of ester *XLII*, m.p. 141 – 144 °C (ether), $[\alpha]_D$ +36° (*c* 0.3, chloroform). IR spectrum (chloroform): 1 819, 1 788 (C=O, imide); 1 743 (C=O, ester); 1 708 (C=O, ketone). For $C_{26}H_{36}N_2O_6$ (472.6) calculated: 66.08% C, 7.68% H, 5.93% N; found: 66.37% C, 7.44% H, 5.67% N.

The authors are indebted to Mrs D. Hybsova for skillful technical assistance and to Dr P. Fiedler for taking and interpretation of IR spectra. Our thanks are due to Mrs M. Snopkova for measurements of the ^1H NMR spectra and Dr D. Saman for taking ^{13}C NMR spectra and help with their interpretation. Mass spectra were measured by the staff of the Laboratory of Mass Spectrometry (Dr K. Ubik, Head), the elemental analyses were carried out in the Analytical Laboratory (Dr V. Pechanec, Head) of this Institute. This work was supported in part from grant No. 203/94/0075 of the Grant Agency of the Czech Republic.

REFERENCES

1. Buzzetti F., Faustini F., Orzi F.: Gazz. Chim. Ital. *115*, 351 (1985).
2. Li T. T., Marx M. (Syntex (U.S.A.) Inc.): U.S. 4,552,898 (1985); Chem. Abstr. *107*, 39269 (1987).
3. Macchia B., Balsamo A., Lapucci A., Macchia F., Martinelli A., Nencetti S., Orlandini E., Baldacci M., Mengozzi G., Soldati G., Domiano P.: J. Med. Chem. *33*, 1423 (1990).
4. Harman N. G., Angelucci F., Suarato A. (Farmitalia, Carlo Erba S.p.A.): Brit. 2,201,419 (1988); Chem. Abstr. *110*, 232022 (1989).
5. Raggio M. L., Watt D. S.: J. Org. Chem. *41*, 1873 (1976).
6. Hosoda H., Sakai Y., Yoshida H., Miyairi S., Ishii K., Nambara T.: Chem. Pharm. Bull. *27*, 742 (1979).
7. Blumbach J., Hammond D. A., Whiting D. A.: J. Chem. Soc., Perkin Trans. 1 *1986*, 261.
8. Patt S. L., Shoolery J. N.: J. Magn. Reson. *46*, 535 (1982).
9. Vasickova S., Pouzar V., Cerny I., Drasar P., Havel M.: Collect. Czech. Chem. Commun. *51*, 90 (1986).
10. Anliker K., Muller M., Wohlfahrt J., Heusser H.: Helv. Chim. Acta *48*, 1404 (1955).