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SYNTHESIS OF MODIFIED VITAMIN D PRECURSORS

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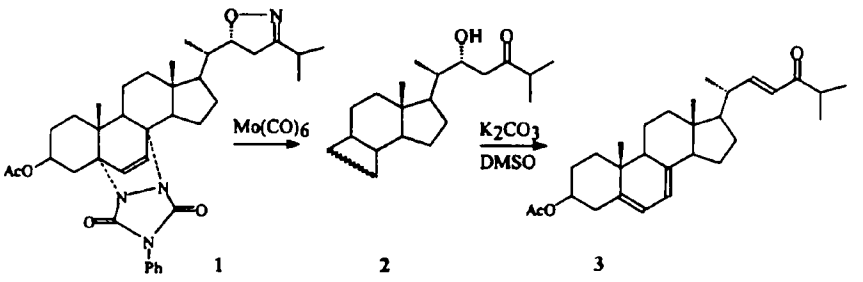
Modified vitamin D precursors with oxygen-containing substituents at various positions in the side-chain were obtained using previously synthesized 20-isoxazolin-3'-yl- and 20-isoxazolin-5'-ylsteroids.

Keywords: isoxazolinylsteroids, polyhydroxysteroids, vitamin D precursors, isoxazoline ring opening.

The rapid progress in the study of new vitamin D analogs has been attributed to the broad spectrum of the biological activity of their active metabolites and the extraordinary importance of analogs with designated activity in practical medicine [1]. The present study on the synthesis of modified vitamin D precursors is a continuation of our work on the synthesis of vitamin D analogs and their metabolites. In previous work [2], we reported the synthesis of a series of vitamin D precursors modified by an isoxazoline ring in the side-chain. In this work, we studied the use of these isoxazolinylsteroids in the synthesis of new analogs of vitamin D precursors with an open side-chain.

Opening of the isoxazoline ring by the action of molybdenum hexacarbonyl [3] was studied in the case of 20-isoxazolin-5'-ylsteroid **1**. Thus, heating **1** with a small excess of molybdenum hexacarbonyl in aqueous acetonitrile for 30 min led to the formation of β-ketol **2** in over 70% yield. Evidence for the structure of **2** was obtained by comparing its spectral characteristics with the data obtained for analogous compounds differing in rings A and B [4]. The signals for the methylene protons at $C_{(24)}$ in the ^{1}H NMR spectrum of **2** are found in the stronger "methylene hump" region and cannot be identified, while the signal for the methine proton at $C_{(22)}$ is shifted upfield relative to cyclic precursor **1**. The shape of the IR signal for the carbonyl group stretching vibrations at 1700-1745 cm^{-1} is complex and a hydroxyl group band is found at 3400 cm^{-1}.



The formation of a small amount of Δ^{22}-24-ketone, which is the product of the dehydration of the secondary hydroxyl group, is observed in addition to β-ketol **2** upon the reductive ring opening of isoxazoline **1** on Raney nickel in the presence of boric acid [4]. Dehydration with elimination of the hydroxyl group at $C_{(22)}$ was also observed upon removal of the triazolidine protection by the action of potassium carbonate in dimethylsulfoxide at

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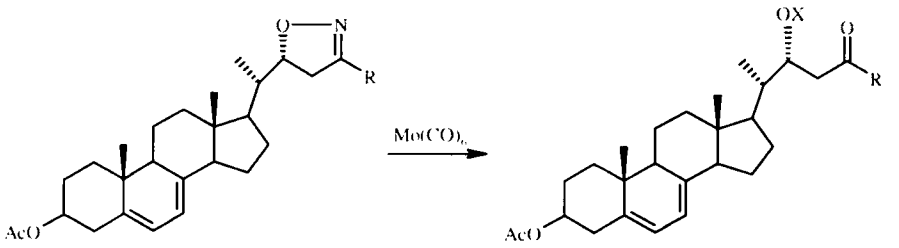
reflux. 5,7,22-Triene-24-ketone **3** was isolated as the major product. The ^{1}H NMR spectrum of **3** features signals for four vinyl protons: one-proton multiplets at 5.40 and 5.58 ppm (protons at $C_{(6)}$ and $C_{(7)}$), a one-proton doublet at 6.08 ppm with J = 16 Hz, and a one-proton doublet of doublets at 6.70 ppm with J_1 = 16 and J_2 = 6 Hz (protons at $C_{(23)}$ and $C_{(22)}$). The IR spectrum of this compound has a band for the stretching vibrations of a conjugated carbonyl group at 1690 cm^{-1} and lacks a hydroxyl group band.

This pathway is a new method for synthesis of a vitamin D precursor with a Δ^{22}-24-keto group in the side-chain, which has been described as in intermediate in the synthesis of active vitamin D analogs [5].

Analogs of vitamin D precursors ($\Delta^{5,7}$-steroids) with a β-ketol group in the side-chain were obtained by an alternative pathway starting from $\Delta^{5,7}$-steroidal isoxazolines **4** and **5** [2]. Opening of the heterocycle in these compounds by the action of molybdenum hexacarbonyl leads to 22-hydroxy-24-ketones **6** and **7**, respectively. The ^{1}H NMR spectra of these products are similar to the spectrum of β-ketol **2** with a one-proton multiplet at 4.13-4.17 ppm characteristic for the proton $C_{(22)}$, but the two one-proton multiplets for the protons at $C_{(6)}$ and $C_{(7)}$ are shifted upfield (5.39 and 5.58 ppm) as found for compounds with a regenerated 5,7-diene group [2]. The signal for the proton at $C_{(3)}$ at 4.71 ppm is also shifted upfield relative to the compound with triazolidine protection. The signal for the hydroxyl group proton for both compounds is a broad one-proton singlet at 3.00 ppm for **6** and 3.10 ppm for **7a**. This signal was identified by shaking a sample of **7a** with D_2O leading to replacement of the hydroxyl protons by deuterium, while there is no hydroxyl proton signal in the ^{1}H NMR spectrum of **7b**. The IR spectra of **6** and **7** show additional signals in the carbonyl group stretching region at 1710 cm^{-1} ($C_{(24)}$ ketone group). Bands for stretching vibrations of the hydroxyl groups of the side-chain are noted at 3450-3510 cm^{-1}.



4 R = Me
5 R = *i*-Pr

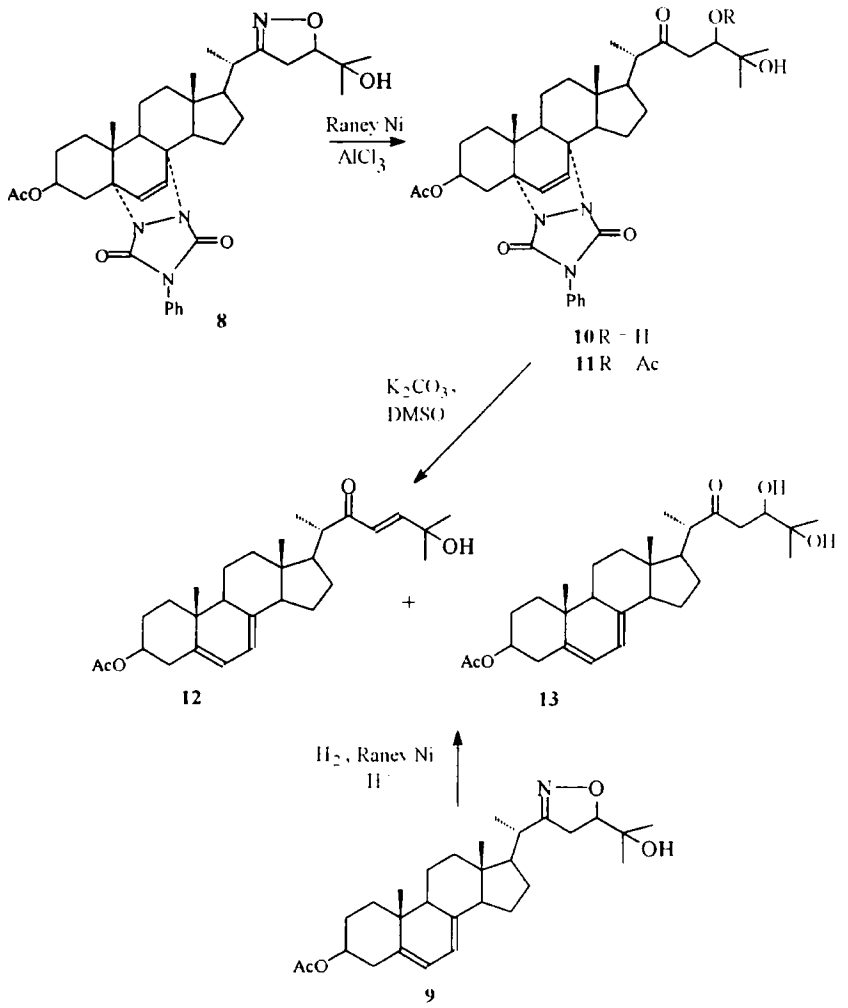
6 R = Me, X = H
7 R = *i*-Pr, X = H(a), X = D(b)

A synthesis of derivatives with a $C_{(25)}$-hydroxyl group characteristic for compounds in the vitamin D group was carried out starting from 20-isoxazolin-3'-ylsteroids **8** and **9** [2]. Opening of the isoxazoline ring of **8** over Raney nickel in the presence of aluminum chloride [6] led to formation of 24,25-dihydroxy-24-ketone **10** with 40% yield. The ^{1}H NMR spectrum of **10** shows a one-proton multiplet at 3.84 ppm related to the proton at $C_{(24)}$ and lacks heterocyclic methylene proton signals. The IR spectrum has a hydroxyl group band at 3450 cm^{-1} and complex absorption in the carbonyl group stretching region (a broad carbonyl group signal is found at 1705 cm^{-1}). Acetylation of the secondary hydroxyl group leads to 3,24-diacetoxy derivative **11**. The ^{1}H NMR of **11** has a second three-proton acetyl group signal, while the signal for the proton at $C_{(24)}$ is shifted downfield to 5.26 ppm.

Regeneration of the 5,7-diene group in order to obtain analogs of vitamin D precursors, as in the case of β-ketol **2**, leads not only to removal of the triazolidine protection, but also to dehydration of the $C_{(24)}$-hydroxyl group. The resultant conjugated ketone **12** has four vinyl proton signals. The signals at 5.39 and 5.58 ppm belong to the protons at $C_{(6)}$ and $C_{(7)}$, while the one-proton doublets at 6.40 and 6.84 ppm, J = 16 Hz, indicate *trans* configuration of the Δ^{23}-bond. The IR spectrum of this compound has bands for the acetyl group carbonyl at 1735 and 1250 cm^{-1}, conjugated ketone at 1695 and 1630 cm^{-1}, and hydroxyl group at 3450 cm^{-1}.

The reductive opening of isoxazoline **9** on Raney nickel led to 24,25-dihydroxy-22-ketone **13** with a 5,7-diene group. Conjugated ketone **12** was obtained as a by-product.

Thus, modified vitamin D precursors with oxygen-containing functions at $C_{(22)}$, $C_{(24)}$, and $C_{(25)}$ in the side-chain were obtained starting from 20-isoxazolinylsteroids. The method developed is simple and convenient and holds promise for the synthesis of other vitamin D precursors and new vitamin D analogs using various isoxazolinylsteroids.



EXPERIMENTAL

The melting points were taken on a Koefler block. The IR spectra were taken on a UR-20 spectrometer. The ^{1}H NMR spectra were taken on a Bruker AC-200 spectrometer at 200 MHz for solutions in $CDCl_3$ with Me_4Si as the internal standard. The mass spectra were taken on a Shimadzu QP-5000 mass spectrometer with direct sample inlet. The temperature was raised from 30 to 350°C at 20°C/min. The ionizing voltage was 70 eV. The reaction course was monitored by thin-layer chromatography on Merck Kieselgel 60 F_{254} plates. Chromatographic separation was carried out on Chemapol 40/100 μm silica gel or Merck Kieselgel 60 (40/60 μm).

Ring Opening in Isoxazolinylsteroids. A. A sample of molybdenum hexacarbonyl (0.044 mmol) was added to a solution of isoxazoline (0.04 mmol) in acetonitrile (5 ml) and water (0.05 ml). The reaction mixture was heated at reflux for 30 min. Silica gel was added and the solvent was distilled off. The residue was subjected to chromatography on a silica gel column using 9:1 toluene–ethyl acetate as the eluent.

B. A sample of Raney nickel (1 g) and anhydrous aluminum chloride (1.125 mmol) were added to a solution of isoxazoline (0.28 mmol) in 5:1 methanol–water (18 ml). The reaction mixture was stirred for 2.5 h. Water was added and the mixture was extracted with ethyl acetate. The extracts were dried over anhydrous sodium sulfate and filtered. The solvent was evaporated. The residue was subjected to chromatography on a silica gel column using 2:1 toluene–ethyl acetate as the eluent.

C. A sample of W-2 Raney nickel was saturated with hydrogen upon stirring in ethanol over 2 h. Then, boric acid (1 mmol) and isoxazolinylsteroid (0.01 mmol) in ethanol were added. The reaction mixture was stirred at room temperature in a hydrogen atmosphere for 5 h. After completion of the reaction as indicated by thin-layer chromatography, the catalyst was filtered off and the solvent was evaporated. The residue was dissolved in ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. The product was purified by chromatography on a silica gel column using 2:7 ether–hexane as the eluent.

(22*R*)-3β-Acetoxy-22-hydroxy-5α,8α-(3',5'-dioxo-4'-phenyl-1',2',4'-triazolidine)cholest-6-en-24-one (2). Procedure A using isoxazoline **1** (0.025 g) gave 0.018 g (72%) of β-ketol **2** as an oil. ^{1}H NMR spectrum: 0.85 (3H, s, 18-CH_3); 0.98 (3H, d, J = 7 Hz, 21-CH_3); 1.01 (3H, s, 19-CH_3); 1.14 (6H, d, J = 7 Hz, 26- and 27-CH_3); 2.02 (3H, s, OAc); 3.23 (1H, dd, J_1 = 15, J_2 = 6 Hz, 9-H); 4.12 (1H, m, 22-H); 5.46 (1H, m, 3-H); 6.26 (1H, d, J = 8 Hz, 6-H); 6.42 (1H, d, J = 8 Hz, 7-H); 7.42 ppm (5H, m, C_6H_5). IR spectrum (neat): 3400, 1745, 1735, 1705 (br); 1250 cm^{-1}. Mass spectrum (*m/z*): 456 [M-$C_8H_5N_1O_2$]$^+$, 396 [M-$C_8H_5N_1O_2$-AcOH]$^+$. Found, %: C 70.52; H 7.80; N 6.60. $C_{37}H_{50}N_4O_6$. Calculated, %: C 70.34; H 7.82; N 6.65.

(22-*R*)-3β-Acetoxy-22-hydroxy-26,27-dinorcholest-5,7-dien-24-one (6). Procedure A using isoxazoline **4** (0.016 g) gave 0.005 g (31%) of β-ketol **6** as an oil. ^{1}H NMR spectrum: 0.65 (3H, s, 18-CH_3); 0.98 (3H, s, 19-CH_3); 1.00 (3H, d, J = 7 Hz, 21-CH_3); 2.05 (3H, s, OAc); 2.23 (3H, s, 25-CH_3); 3.00 (1H, br. s, OH); 4.17 (1H, m, 22-H); 4.71 (1H, m, 3-H); 5.39 (1H, m, 6-H); 5.58 ppm (1H, m, 7-H). IR spectrum (neat): 3510, 1730, 1710, 1255 cm^{-1}. Mass spectrum (*m/z*): 428 [M]$^+$, 368 [M-AcOH]$^+$. Found, %: C 75.50; H 9.35. $C_{27}H_{40}O_4$. Calculated, %: C 75.66; H 9.41.

(22*R*)-3β-Acetoxy-22-hydroxycholest-5,7-dien-24-one (7a). Procedure A using isoxazoline **5** (0.014 g) gave 0.007 g (50%) of β-ketol **7a** as an oil. ^{1}H NMR spectrum: 0.65 (3H, s, 18-CH_3); 0.96 (3H, s, 19-CH_3); 0.98 (3H, d, J = 7 Hz, 21-CH_3); 1.13 (6H, d, J = 7 Hz, 26- and 27-CH_3); 2.04 (3H, s, OAc); 3.10 (1H, br. s, OH); 4.13 (1H, m, 22-H); 4.71 (1H, m, 3-H); 5.39 and 5.58 ppm (2H, two m, 6- and 7-H). IR spectrum (neat): 3450, 1730, 1710, 1255 cm^{-1}. Mass spectrum (*m/z*): 456 [M]$^+$, 396 [M-AcOH]$^+$. Found, %: C 76.32; H 9.60. $C_{29}H_{44}O_4$. Calculated, %: C 76.27; H 9.71.

Shaking with D_2O gave deuterated analog **7b**. ^{1}H NMR spectrum: 0.65 (3H, s, 18-CH_3); 0.96 (3H, s, 19-CH_3); 0.98 (3H, d, J = 7 Hz, 21-CH_3); 1.13 (6H, d, J = 7 Hz, 26- and 27-CH_3); 2.04 (3H, s, OAc); 4.13 (1H, m, 22-H); 4.71 (1H, m, 3-H); 5.39 and 5.58 ppm (2H, two m, 6- and 7-H).

(24ξ)-3β-Acetoxy-24,25-dihydroxy-5α,8α-(3',5'-dioxo-4'-phenyl-1',2',4'-triazolidine)cholest-6-en-22-one (10). Procedure B using isoxazolidine **8** (0.18 g) gave 0.072 g (40%) of 24,25-dihydroxy-22-ketone **10** as an oil. ^{1}H NMR spectrum: 0.84 (3H, s, 18-CH_3); 1.00 (3H, s, 19-CH_3); 1.19 and 1.21 (6H, two s, 26- and 27-CH_3); 1.24 (3H, d, J = 7 Hz, 21-CH_3); 2.04 (3H, s, OAc); 3.23 (1H, dd, J_1 = 14, J_2 = 5 Hz, 9-H); 3.84 (1H, m, 24-H); 5.46 (1H, m, 3-H); 6.25 and 6.40 (2H, two d, J = 8 Hz, 6- and 7-H); 7.42 ppm (5H, m, C_6H_5). IR spectrum (neat): 3450, 1755, 1740, 1705 (br); 1250 cm^{-1}. Mass spectrum (*m/z*): 472 [M-$C_8H_5N_1O_2$]$^+$, 412 [M-$C_8H_5N_1O_2$-AcOH]$^+$. Found, %: C 68.50; H 7.53; N 6.43. $C_{37}H_{50}N_4O_7$. Calculated, %: C 68.59; H 7.62; N 6.49.

(24ξ)-3β-Acetoxy-24,25-dihydroxycholest-5,7-dien-22-one (13). Procedure C using isoxazoline **9** (0.047 g) gave 0.007 g (15%) of unsaturated ketone **12** and 0.024 g (50%) of diolketone **13** as an oil. ^{1}H NMR spectrum: 0.68 (3H, s, 18-CH_3); 1.00 (3H, s, 19-CH_3); 1.20 (6H, s, 26- and 27-CH_3); 1.24 (3H, d, J = 7 Hz, 21-CH_3); 2.05 (3H, s, OAc); 3.23 (1H, dd, J_1 = 14, J_2 = 5 Hz, 9-H); 3.85 (1H, m, 24-H); 5.46 (1H, m, 3-H); 5.40 and 5.60 ppm (2H, two d, J = 8 Hz, 6- and 7-H). IR spectrum (neat): 3450, 1735, 1705, 1250 cm^{-1}. Mass spectrum (*m/z*): 472 [M]$^+$, [M-AcOH]$^+$, 352 [M-AcOH-H_2O]$^+$. Found, %: C 73.52; H 9.29. $C_{29}H_{44}O_5$. Calculated, %: C 73.69; H 9.38.

Removal of the Triazolidine Protection. A sample of anhydrous potassium carbonate (0.075 mmol) was added to a solution of triazolidine derivative (0.062 mmol) in dimethylsulfoxide (3.5 ml). The reaction mixture was maintained for 7 h in an nitrogen stream at 120°C. After cooling, the solution was neutralized by adding 0.5% hydrochloric acid and extracted with ethyl acetate. The extract was washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was subjected to chromatography on a silica gel column using 7:1 toluene–ethyl acetate as the eluent.

(22ξ)-3β-Acetoxycholest-5,7,22-trien-24-one (3). This procedure using β-ketol **2** (0.039 g) gave 0.020 g (74%) of 5,7,22-triene-24-one **3**; mp 133-136°C (CH_2Cl_2–MeOH). ^{1}H NMR spectrum: 0.83 (3H, s, 18-CH_3);

1.00 (3H, s, 19-CH_3); 0.96 (3H, d, J = 7 Hz, 21-CH_3); 1.10 (6H, s, 26- and 27-CH_3); 2.05 (3H, s, OAc); 2.84 (3H, s, 25-H); 4.71 (1H, m, 3-H); 5.40 and 5.58 (2H, two m, 6- and 7-H); 6.08 (1H, d, J = 16 Hz, 23-H); 6.70 ppm (1H, dd, J_1 = 16, J_2 = 6 Hz, 22-H). IR spectrum (neat): 1735, 1695, 1630, 1250 cm^{-1}. Mass spectrum (m/z): 438 $[M]^+$, 378 $[M-AcOH]^+$. Found, %: C 79.42; H 9.60. $C_{30}H_{42}O_5$. Calculated, %: C 79.40; H 9.65.

(22ξ)-3β-Acetoxy-25-hydroxycholest-5,7,23-trien-22-one (12). This procedure using triazolidine derivative **10** (0.04 g) gave 0.021 g (75%) of 5,7,23-triene-22-ketone **12**; mp 144-146°C (CH_2Cl_2–MeOH). ^1H NMR spectrum: 0.66 (3H, s, 18-CH_3); 0.97 (3H, s, 19-CH_3); 1.18 (3H, d, J = 7 Hz, 21-CH_3); 1.40 (6H, s, 26- and 27-CH_3); 2.05 (3H, s, OAc); 4.71 (1H, m, 3-H); 5.39 and 5.58 (2H, two m, 6- and 7-H); 6.40 and 6.84 ppm (2H, two d, J = 16 Hz, 23- and 24-H). IR spectrum (neat): 3450, 1735, 1695, 1670, 1630, 1250 cm^{-1}. Mass spectrum (m/z): 454 $[M]^+$, 394 $[M-AcOH]^+$. Found, %: C 76.42; H 9.28. $C_{30}H_{42}O_4$. Calculated, % C 76.61; H 9.31.

Acetylation of Secondary Alcohol (24ξ)-3β,24-Diacetoxy-25-hydroxy-5α,8α-(3',5'-dioxo-4'-phenyl-1',2',4'-triazolidine)cholest-6-en-22-one (11). A sample of steroid alcohol **10** (0.012 g, 0.02 mmol) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.25 ml) was added. The reaction mixture was left for 18-20 h, treated with water (10 ml), and extracted with ether. The extract was washed with 0.5% hydrochloric acid until neutral and dried over anhydrous sodium sulfate. The solvent was evaporated. The residue was dissolved in a small amount of chloroform and purified by passing through a layer of silica gel to give 0.010 g (92%) of 24-acetoxy derivative **11** as an oil. ^1H NMR spectrum: 0.84 (3H, s, 18-CH_3); 1.00 (3H, s, 19-CH_3); 1.20 (3H, d, J = 7 Hz, 21-CH_3); 1.23 (6H, d, J = 7 Hz, 26- and 27-CH_3); 2.04 and 2.05 (6H, two s, two OAc); 3.24 (1H, dd, J_1 = 14, J_2 = 5 Hz, 9-H); 5.26 (1H, m, 24-H); 5.46 (1H, m, 3-H); 6.25 and 6.40 (2H, two d, J = 8 Hz, 6- and 7-H); 7.42 ppm (5H, m, C_6H_5). IR spectrum (neat): 3450, 1755 (br); 1710 (br); 1250 cm^{-1}. Mass spectrum (m/z): 689 $[M]^+$, 514 $[M-C_8H_5N_3O_2]^+$, 454 $[M-C_8H_5N_3O_2-AcOH]^+$, 394 $[M-C_8H_5N_3O_2-2AcOH]^+$. Found, %: C 67.76; H 7.43; N 6.00. $C_{44}H_{51}N_3O_8$. Calculated, %: C 67.90; H 7.45; N 6.09.

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