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Synthesis of Estriol Monoglucoside Derivatives, New Haptens for Production of Specific Antisera[1]

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For the purpose of obtaining antisera used for direct radioimmunoassay of estriol glucuronides, two new haptens, estriol 3- and 16-glucoside derivatives possessing a carboxyl group, have been synthesized. Condensation of estriol 3-carboxymethyl ether with 1-bromo-1-deoxy-α-D-glucopyranose tetraacetate in the presence of cadmium carbonate provided the 16-glucoside together with a small amount of the 17-isomer. The Koenigs–Knorr reaction with 6-oxoestriol 16,17-diacetate under similar conditions afforded the 3-glucoside which in turn was led to the 6-(O-carboxymethyl)oxime. Removal of the protecting groups in estriol 3- and 16-glucoside derivatives was effected by treatment with methanolic alkali to yield the desired compounds.

Keywords——radioimmunoassay; *anti*-estriol glucuronide antisera; heterologous combination; hapten; Koenigs–Knorr reaction; cadmium carbonate catalyst; estriol 16-glucoside; 6-oxoestriol 3-glucoside

In recent years considerable attention has been focused to the development of direct radioimmunoassay of the steroid glucuronide without prior hydrolysis.[3–7] However, almost all attempts for obtaining the specific antisera to the conjugated steroid resulted in failure. This might be attributable to the structure of the hapten-carrier conjugate where the carboxyl group in the glucuronyl moiety was used for linking to a carrier protein. We have attempted to utilize a heterologous combination of antigen and antibody. It is supposed that the anti-steroid glucoside antibody may possibly show the immune reactivity toward the steroid glucuronide antigen. The present paper deals with the synthesis of estriol (1,3,5(10)-estratriene-3,16α,17β-triol) 3- and 16-glucoside derivatives having a carboxyl group which may serve as haptens for production of the specific antisera to estriol monoglucuronides.

An initial project was directed to the preparation of estriol 16-glucoside 3-carboxymethyl ether. Bernstein and his co-workers[8] have developed a convenient method to obtain the 16-glucuronide by introducing a glucuronyl moiety directly into the C-16 position of estriol 3-benzyl ether. Accordingly, estriol (**1**) was converted by treatment with monobromoacetic acid and sodium *n*-propoxide in *n*-propanol into the 3-carboxymethyl ether which on methylation with diazomethane was led to the methyl ester (**2**). The Koenigs–Knorr reaction with 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose in the presence of cadmium carbonate as a catalyst[9,10] furnished two isomeric glucoside derivatives (**3**,**4**) in a ratio of *ca.* 4 to 1,

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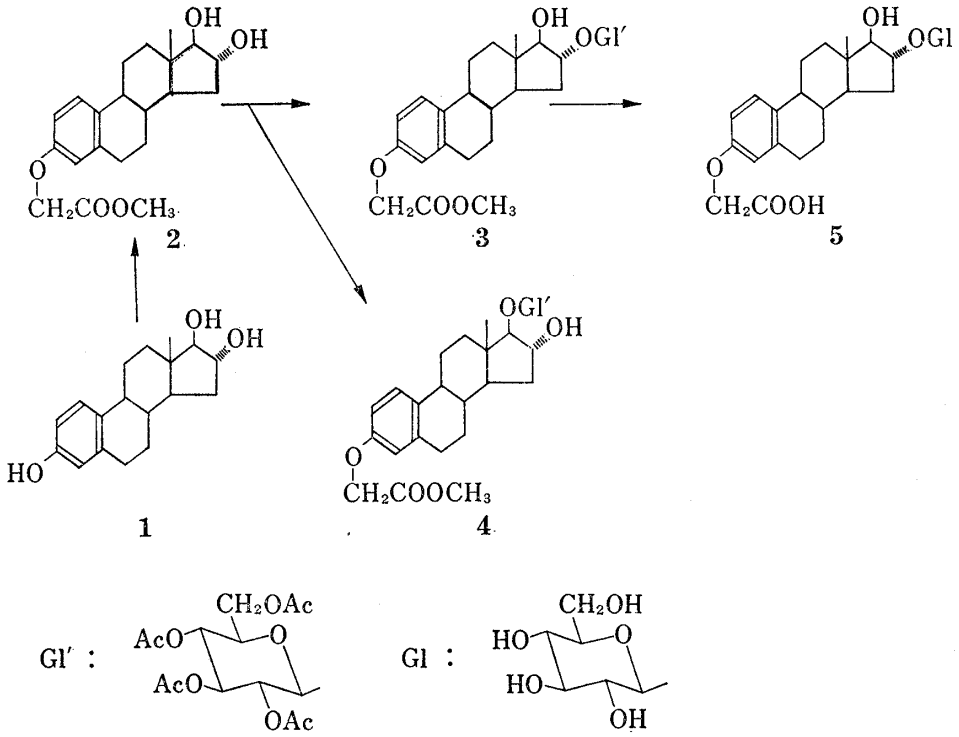

Chart 1

whose separation was attained by preparative thin-layer chromatography (TLC). Inspection of their nuclear magnetic resonance (NMR) spectra permitted us to assign the structures to these positional isomers. As listed in Table I the chemical shifts of 18-methyl, 16- and 17-protons in the isomeric glucosides were in good accord with those observed with estriol ring D glucuronides, respectively. The present result on the reactivities of hydroxyl groups at

Table I. Nuclear Magnetic Resonance Spectral Data of Estriol Ring D Glucoside and Glucuronide Derivatives

Compound[a]	Chemical shift (δ) ppm		
	18-CH_3	16β-H	17α-H
16-Gl'(3)	0.80	3.80—4.15[b]	3.66
17-Gl'(4)	0.77	4.24	3.33
16-G'[c]	0.81	3.89	3.67
17-G'[c]	0.78	4.00—4.40[b]	3.32

a) Gl': β-D-glucopyranoside tetraacetate; G': β-D-glucopyranuronoside triacetate-methyl ester.
b) Overlapped with signals due to pyranose-C_5-H.
c) T. Nambara, Y. Kawarada, K. Shibata, and T. Abe, *Chem. Pharm. Bull.* (Tokyo), **20**, 1988 (1972).

C-16 and C-17 with the acetobromosugar is consistent with the previous finding.[8] Simultaneous removal of the protecting groups in both sugar and steroid moieties in **3** was effected by exposure to methanolic sodium hydroxide to provide the desired estriol 16-β-D-glucopyranoside 3-carboxymethyl ether (**5**) in a satisfactory yield.

Next effort was focused to the synthesis of estriol 3-glucoside derivative having a carboxyl group for conjugation with a carrier protein. For this purpose 6-oxoestriol triacetate (**6**), which is available in two steps from estriol,[11] was taken as a starting compound. Partial

11) C.S. Walker, S.J. Clark, and H.H. Wotiz, *Steroids*, **21**, 259 (1973).

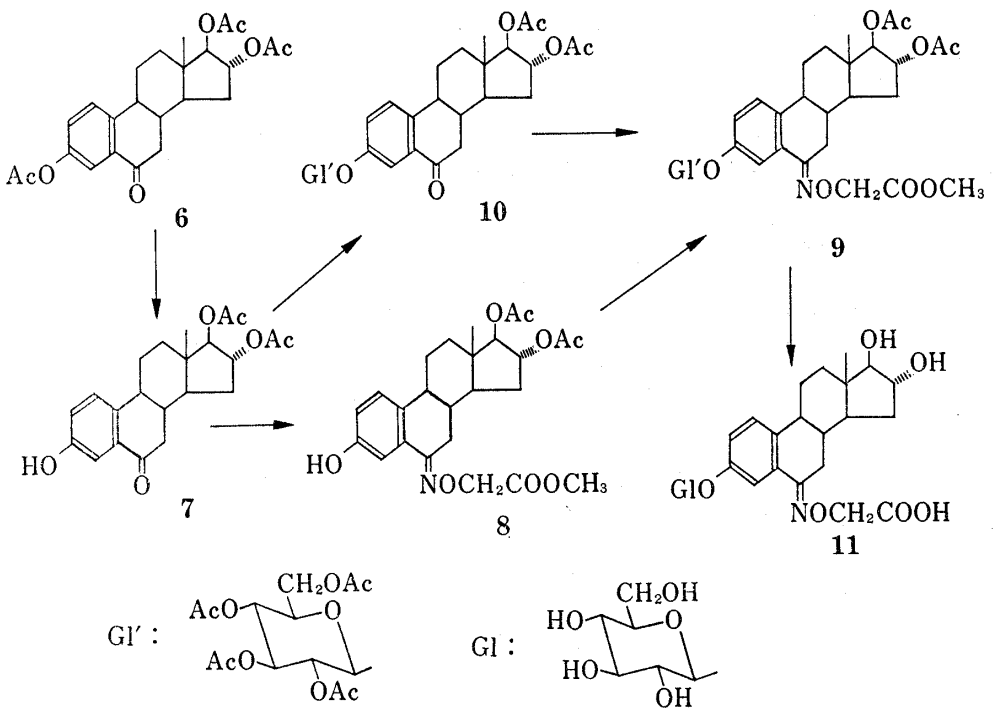

Chart 2

hydrolysis of the acetoxyl group at C-3 with potassium bicarbonate under the mild condition yielded the 16,17-diacetate (**7**). Condensation with carboxymethylhydroxyamine, followed by methylation with diazomethane furnished the 6-oxime derivative (**8**) as an oily product. As judged from the NMR spectral data the product appeared to be a mixture of the *syn* and *anti* forms. Condensation of **8** with the acetobromosugar in the presence of cadmium carbonate in anhydrous toluene proceeded readily to afford the 3-glucoside tetraacetate (**9**). The preparation of this compound was carried out by an alternative route involving the reversed reaction sequence. The Koenigs-Knorr reaction with **7** in the manner as described above furnished 6-oxoestriol 3-glucoside derivative (**10**). Subsequent treatment with carboxymethylhydroxyamine and diazomethane yielded the oxime derivative (**9**) as a sole product. The stereoselective attack of the reagent toward the 6-oxo group may probably be due to the presence of the bulky substituent at C-3. Elimination of the protecting groups in **9** with methanolic alkali provided 6-oxoestriol 3-β-D-glucopyranoside (O-carboxymethyl)oxime (**11**) in a reasonable yield.

The NMR spectra of estriol glucoside tetraacetates were indicative of the formation of the β-glucopyranoside structure. The anomeric proton in the sugar moiety appeared at 4.56 or 5.20 ppm as a doublet (J=7 Hz) indicating a *trans*-diaxial relationship to the vicinal 2'-proton.

The preparation and antigenic properties of the hapten-bovine serum albumin conjugates will be the subject of a future communication.

Experimental[12]

1,3,5(10)-Estratriene-3,16α,17β-triol 3-Methoxycarbonylmethyl Ether (**2**)——To a solution of 1,3,5(10)-estratriene-3,16α,17β-triol (**1**) (1 g) in anhydrous *n*-PrOH (35 ml) was added metal Na (1.4 g) and refluxed

12) All melting points were taken on a micro hot-stage apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$. NMR spectra were recorded on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as an internal standard. Abbreviation used s=singlet, d=doublet, q=quartet, and m=multiplet. For preparative TLC silica gel HF_{254} (E. Merck AG, Darmstadt) was used as an adsorbent.

for 10 min. To this solution was added monobromoacetic acid (4 g) in anhydrous *n*-PrOH (5 ml), refluxed for 13 hr, and then concentrated *in vacuo*. To the resulting solution was added 2 N Na_2CO_3 and extracted with AcOEt for removal of the starting material. The aqueous layer was adjusted to pH 2 with 5% HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was treated with CH_2N_2 in the usual manner. The crude product was submitted to column chromatography on silica gel (25 g). Elution with cyclohexane–AcOEt (1: 4) and recrystallization of the eluate from acetone–hexane gave **2** (527 mg) as colorless plates. mp 136—138°. $[\alpha]_D^{17}$ +45.6° (c=0.10). *Anal.* Calcd. for $C_{21}H_{28}O_5$: C, 69.97; H, 7.83. Found: C, 69.74; H, 7.92. NMR ($CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 3.60 (1H, d, J=6 Hz, 17α-H), 3.80 (3H, s, -$COOCH_3$), 4.20 (1H, m, 16β-H), 4.60 (2H, s, -OCH_2–), 6.63 (1H, s, 4-H), 6.68 (1H, q, J=2, 8 Hz, 2-H), 7.17 (1H, d, J=8 Hz, 1-H).

Koenigs-Knorr Reaction of 2——To a solution of **2** (360 mg) in anhydrous toluene (37 ml) were added 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (300 mg) in anhydrous toluene (4 ml) and freshly prepared $CdCO_3$ (740 mg) and refluxed for 2 hr. An additional portion of the acetobromosugar (220 mg) in anhydrous toluene (4 ml) was then added and refluxed for another 4 hr. The precipitate was removed by filtration and the filtrate was concentrated. An oily residue obtained was chromatographed on silica gel (12 g) and eluted with cyclohexane–AcOEt (2: 1). The eluate was submitted to preparative TLC using benzene–ether (2: 1) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.65) and recrystallization of the eluate from acetone–hexane gave 3,17β-dihydroxy-1,3,5(10)-estratrien-16α-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside 3-methoxycarbonylmethyl ether (**3**) (130 mg) as colorless needles. mp 172—174°. $[\alpha]_D^{17}$ −104.7° (c=0.05). *Anal.* Calcd. for $C_{35}H_{46}O_{14}$: C, 60.86; H, 6.71. Found: C, 60.56; H, 6.63. NMR ($CDCl_3$) δ: 0.80 (3H, s, 18-CH_3), 2.03, 2.07, 2.15 (12H, s, -$OCOCH_3$), 3.66 (1H, d, J=5 Hz, 17α-H), 3.81 (3H, s, -$COOCH_3$), 3.80—4.15 (2H, m, 16β-H and pyranose-C_5-H), 4.56 (1H, d, J=7 Hz, pyranose-C_1-H), 4.60 (2H, s, -OCH_2–), 4.90—5.38 (5H, m, pyranose-CH-OAc), 6.63 (1H, s, 4-H), 6.68 (1H, q, J=2, 8 Hz, 2-H), 7.18 (1H, d, J=8 Hz, 1-H).

Elution of the adsorbent corresponding to the spot (*Rf* 0.60) and recrystallization of the eluate from acetone–hexane gave 3,16α-dihydroxy-1,3,5(10)-estratrien-17β-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside 3-methoxycarbonylmethyl ether (**4**) (32 mg) as colorless plates. mp 209—211°. $[\alpha]_D^{17}$ +59.7° (c=0.09). *Anal.* Calcd. for $C_{35}H_{46}O_{14}$: C, 60.86; H, 6.71. Found: C, 60.70; H, 6.54. NMR ($CDCl_3$) δ: 0.77 (3H, s, 18-CH_3), 2.05, 2.08, 2.10, 2.13 (12H, s, -$OCOCH_3$), 3.33 (1H, d, J=5 Hz, 17α-H), 3.82 (3H, s, -$COOCH_3$), 3.80–4.05 (1H, m, pyranose-C_5–H), 4.24 (1H, m, 16β-H), 4.57 (1H, d, J=7 Hz, pyranose-C_1–H), 4.61 (2H, s, -OCH_2–), 4.90—5.40 (5H, m, pyranose-CH-OAc), 6.64 (1H, s, 4-H), 6.68 (1H, q, J=2, 8 Hz, 2-H), 7.16 (1H, d, J=8 Hz, 1-H).

3,17β-Dihydroxy-1,3,5(10)-estratrien-16α-yl-β-D-glucopyranoside 3-Carboxymethyl Ether (5)——**3** (50 mg) was dissolved in 2 N methanolic NaOH (3 ml) and allowed to stand at room temperature overnight. The resulting solution was concentrated to a small volume, diluted with H_2O, and passed through a column of Amberlite XAD-2 resin (10 mm × 15 cm). After usual work-up the crude product obtained was recrystallized from MeOH to give Na salt of **5** (15 mg) as colorless plates. mp 260° (dec.). *Anal.* Calcd. for $C_{26}H_{35}NaO_{10}\cdot 3/2H_2O$: C, 56.00; H, 6.87. Found: C, 55.89; H, 6.73.

3,16α,17β-Trihydroxy-1,3,5(10)-estratrien-6-one 16,17-Diacetate (7)——To a solution of 3,16α,17β-trihydroxy-1,3,5(10)-estratrien-6-one triacetate (**6**) (400 mg) in EtOH (20 ml) was added 30% $KHCO_3$ solution (2 ml) and stirred at 35° for 15 hr. The resulting solution was poured onto ice-water and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. Recrystallization from acetone–hexane gave **7** (350 mg) as colorless plates. mp 240—242°. $[\alpha]_D^{17}$ +84.4° (c=0.10). *Anal.* Calcd. for $C_{22}H_{26}O_6$: C, 68.38; H, 6.78. Found: C, 68.24; H, 6.75. NMR ($CDCl_3$) δ: 0.86 (3H, s, 18-CH_3), 2.06, 2.11 (6H, s, -$OCOCH_3$), 5.00 (1H, d, J=5 Hz, 17α-H), 5.22 (1H, m, 16β-H), 7.15 (1H, q, J=2, 8 Hz, 2-H), 7.20 (1H, d, J=8 Hz, 1-H), 7.64 (1H, d, J=2 Hz, 4-H).

16α,17β-Diacetoxy-3-hydroxy-1,3,5(10)-estratrien-6-one O-(Methoxycarbonylmethyl)oxime (8)——To a solution of **7** (300 mg) in *n*-PrOH (60 ml) were added O-carboxymethylhydroxyamine·HCl (500 mg) and AcONa (800 mg) in H_2O (20 ml) and refluxed for 4 hr. The resulting solution was concentrated *in vacuo*, diluted with 2 N Na_2CO_3, and extracted with AcOEt for removal of the starting material. The aqueous layer was acidified with conc. HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated. An oily residue obtained was treated with CH_2N_2 in the usual manner. The crude product was submitted to column chromatography on silica gel (10 g). Elution with cyclohexane–AcOEt (5: 1) gave **8** (248 mg) as pale yellow oily substance. NMR (CCl_4) δ: 0.77 (3H, s, 18-CH_3), 2.03, 2.06 (6H, s, -$OCOCH_3$), 3.85 (3H, s, -$COOCH_3$), 4.67 (2H, s, -OCH_2–), 4.86 (1H, d, J=5 Hz, 17α-H), 5.14 (1H, m, 16β-H), 6.72 (1H, m, 2-H), 6.90 (1H, m, 1-H), 7.13, 7.30 (1H, broad s, 4-H).

16α,17β-Diacetoxy-6-oxo-1,3,5(10)-estratrien-3-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (10)——To a solution of **7** (290 mg) in anhydrous toluene (20 ml) were added 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (400 mg) in anhydrous toluene (2 ml) and freshly prepared $CdCO_3$ (580 mg) and refluxed for 7 hr. An additional portion of the acetobromosugar (300 mg) in anhydrous toluene (2 ml) and $CdCO_3$ (300 mg) were then added and refluxed for another 10 hr. The precipitate was removed by filtration and the filtrate was concentrated. An oily residue obtained was chromatographed on silica gel (10 g) and eluted with cyclohexane–AcOEt (3: 1). The eluate was submitted to preparative TLC using cyclohexane–AcOEt

(2: 3) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.60) and recrystallization of the eluate from acetone–hexane gave **10** (120 mg) as colorless needles. mp 192—194°. $[\alpha]_D^{17}$ −92.7° (c=0.10). *Anal.* Calcd. for $C_{36}H_{44}O_{15}$: C, 60.33; H, 6.19. Found: C, 60.03; H, 6.26. NMR ($CDCl_3$) δ: 0.85 (3H, s, 18-CH_3), 2.06, 2.11, 2.17 (18H, s, -OCOCH_3), 3.81 (3H, s, -COOCH_3), 3.80—4.00 (1H, m, pyranose-C_5-H), 5.02 (1H, d, J=5 Hz, 17α-H), 5.20 (1H, d, J=7 Hz, pyranose-C_1-H), 5.10—5.50 (6H, m, 16β-H, pyranose-CH-OAc), 7.20 (1H, q, J=2, 8 Hz, 2-H), 7.40 (1H, d, J=8 Hz, 1-H), 7.75 (1H, d, J=2 Hz, 4-H).

16α,17β-Diacetoxy-6-oxo-1,3,5(10)-estratrien-3-yl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside O-(Methoxycarbonylmethyl)oxime (9)——i) To a solution of **10** (60 mg) in *n*-PrOH (12 ml) were added O-carboxymethylhydroxyamine·HCl (100 mg) and AcONa (190 mg) in H_2O (5.2 ml) and refluxed for 2 hr. The resulting solution was treated in the manner as described in **8** and an oily residue obtained was chromatographed on silica gel (5 g). Elution with hexane–AcOEt (1: 1) and recrystallization of the eluate from acetone–hexane gave **9** (25 mg) as colorless plates. mp 172—173°. $[\alpha]_D^{17}$ −135.7° (c=0.05). *Anal.* Calcd. for $C_{39}H_{49}NO_{17}$: C, 58.27; H, 6.14; N, 1.74. Found: C, 58.06; H, 6.04; N, 1.83. NMR ($CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 2.05, 2.08, 2.11 (18H, s, -OCOCH_3), 3.81 (3H, s, -COOCH_3), 3.80—4.00 (1H, m, pyranose-C_5-H), 4.76 (2H, s, -OCH_2-), 5.02 (1H, d, J=5 Hz, 17α-H), 5.20 (1H, d, J=7 Hz, pyranose-C_1-H), 5.10—5.50 (6H, m, 16β-H, pyranose-CH-OAc), 7.00 (1H, q, J=2, 8 Hz, 2-H), 7.27 (1H, d, J=8 Hz, 1-H), 7.59 (1H, d, J= 2 Hz, 4-H).

ii) To a solution of **8** (220 mg) in anhydrous toluene (16 ml) were added 1-bromo-1-deoxy-2,3,4,6-tetra-O-acetyl-α-D-glucopyranose (320 mg) in anhydrous toluene (2 ml) and freshly prepared $CdCO_3$ (400 mg) and refluxed for 7 hr. After removal of the precipitate by filtration the filtrate was concentrated. An oily residue obtained was chromatographed on silica gel (10 g) and eluted with cyclohexane–AcOEt (3: 1). The eluate was submitted to preparative TLC using cyclohexane–AcOEt (2: 3) as developing solvent. Elution of the adsorbent corresponding to the spot (*Rf* 0.55) and recrystallization of the eluate from acetone–hexane gave **9** (78 mg) as colorless plates. mp 171—173°. Mixed melting point on admixture with the sample obtained in i) showed no depression.

16α,17β-Dihydroxy-6-oxo-estra-1,3,5(10)-trien-3-yl-β-D-glucopyranoside O-(Carboxymethyl)oxime (11)——**9** (53 mg) was dissolved in 2 N methanolic NaOH (1.8 ml) and allowed to stand at room temperature overnight. The resulting solution was treated in the manner as described in **5** and the crude product obtained was recrystallized from EtOH to give Na salt of **11** (17 mg) as colorless amorphous substance. mp 215—218° (dec.). *Anal.* Calcd. for $C_{26}H_{34}NNaO_{11}\cdot H_2O$: C, 54.07; H, 6.28; N, 2.43. Found: C, 53.97; H, 6.38; N, 2.02.

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