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CEREBROSIDE ANALOGUES FROM 3-PHENYLSERINES

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Cerebroside analogues were synthesized from DL-*threo*-and DL-*erythro*-3-phenylserines by the following sequence of reactions: esterification, N-acylation, reduction with sodium bis (2-methoxyethoxy)-aluminum hydride (SMEAH), and condensation with acetobromoglucose followed by deacetylation. Mass spectrometry disclosed that the glycosidic bond was formed at the primary hydroxyl group.

I. Introduction

Relatively little research has been done in the area of lipid antimetabolites [1–5]. Since cerebrosides are universal constituents of animal cell membranes, the preparation of cerebroside analogues was undertaken to study their effects on rat brain cerebroside galactosidase in vitro [2] and on cerebroside synthesis in vivo. It is hoped that the synthesis of these compounds will yield information on the processes that regulate cerebroside metabolism and methods for their control, be useful in the lysosomal storage diseases such as the sphingolipidoses, be of value in anticonvulsant therapy, and act as potential antitumor agents by selectively interfering with the biosynthesis and/or permeability of the cell membranes of pathological cells.

A cerebroside is composed of a fatty acid in amide linkage and a sugar, usually glucose or galactose, in β-glycosidic linkage on the primary hydroxyl group of the long chain base, sphingenine, D-*erythro*-1,3-dihydroxy-2-amino-*trans*-4-octadecene [5]. Because of the steric similarity in functional groups to the natural base, DL-*erythro*-3-phenyl-1,3-dihydroxy-2-aminopropane was selected as the building block for the synthesis of the cerebroside analogues. This compound had been chosen earlier for the preparation of fatty acid amides as potential inhibitors of rat brain cerebroside galactosidase [6]. Since DL-*erythro*-3-phenyl-1,3-dihydroxy-2-aminopropane was not commercially available, its synthesis was attempted from DL-*erythro*-3-phenylserine. This acid appeared to be an appropriate starting material because the secondary hydroxyl group could be protected during the reaction se-

quence via an oxazoline intermediate. It was necessary to have the secondary hydroxyl group in the cerebroside analogues free because the allylic hydroxyl group in cerebroside is free. In exploratory runs, DL-*threo*-3-phenylserine was used first because it was easier to prepare and could also be purchased. The intended sequence of reactions was (1) esterification to the ethyl ester hydrochloride; (2) liberation of the free base ester; (3) reaction with benzimido ethyl ether hydrochloride to yield DL-*threo*-2,5-diphenyl-4-carboethoxy-2-oxazoline; and (4) reduction with sodium bis(2-methoxyethoxy)-aluminum hydride (SMEAH) to give DL-*threo*-2,5-diphenyl-4-hydroxy methyl-2-oxazoline (fig. 1). Unfortunately, this compound failed to yield a glycoside upon reaction with acetobromoglucose. Although condensation occurred, decomposition of the product resulted during purification. In addition, the oxazoline ring resisted opening even after treatment with 6N HCl. Hydrogenation over platinum in ethanol yielded a mixture of products, one of which was ninhydrin positive

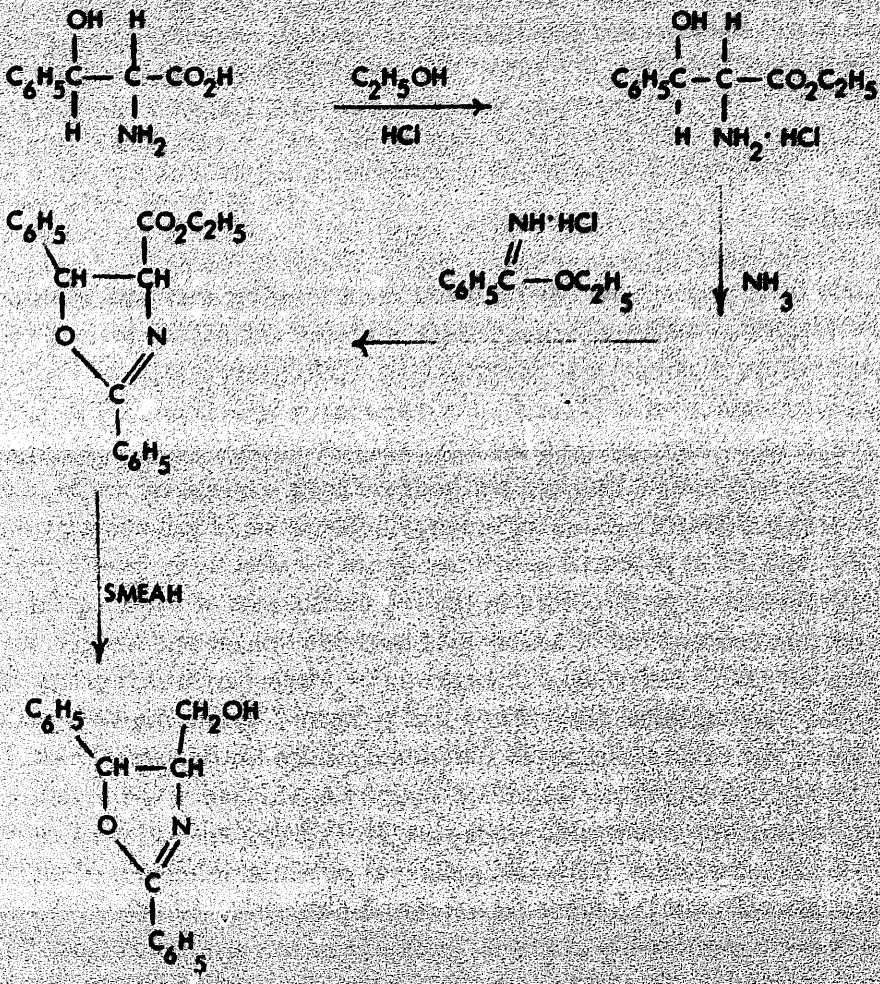

Fig. 1. Sequence of reactions for the synthesis of DL-*threo*-2,5-diphenyl-4-hydroxymethyl-2-oxazoline from DL-*threo*-3-phenylserine. See text for details.

and which indicated that ring opening had occurred. This mixture was not characterized further. It was thought that the phenyl substituents stabilized the oxazoline ring and made the oxygen of the hydroxymethyl group more positive by electron withdrawal. When DL-*threo*-2-methyl-4-hydroxymethyl-5-phenyl-2-oxazoline was prepared scission of the ring was easily accomplished. However, the study of this compound was not pursued further because of the low yields.

Since the secondary hydroxyl group of DL-*erythro*-3-phenyl-1,3-dihydroxy-2-aminopropane is considerably less reactive than the primary one as determined by the vigorous conditions necessary to form the triacetyl derivative [7], an alternative sequence of reactions was chosen for the preparation of the cerebroside analogues in which DL-*threo*-3-phenylserine was (1) esterified to the ethyl ester hydrochloride; (2) *N*-acylated; (3) reduced with SMEAH; and (4) condensed with acetobromoglucose followed by deacetylation (fig. 2). After purification by column chromatography, the product showed a single component on thin layer chromatography (TLC) and the

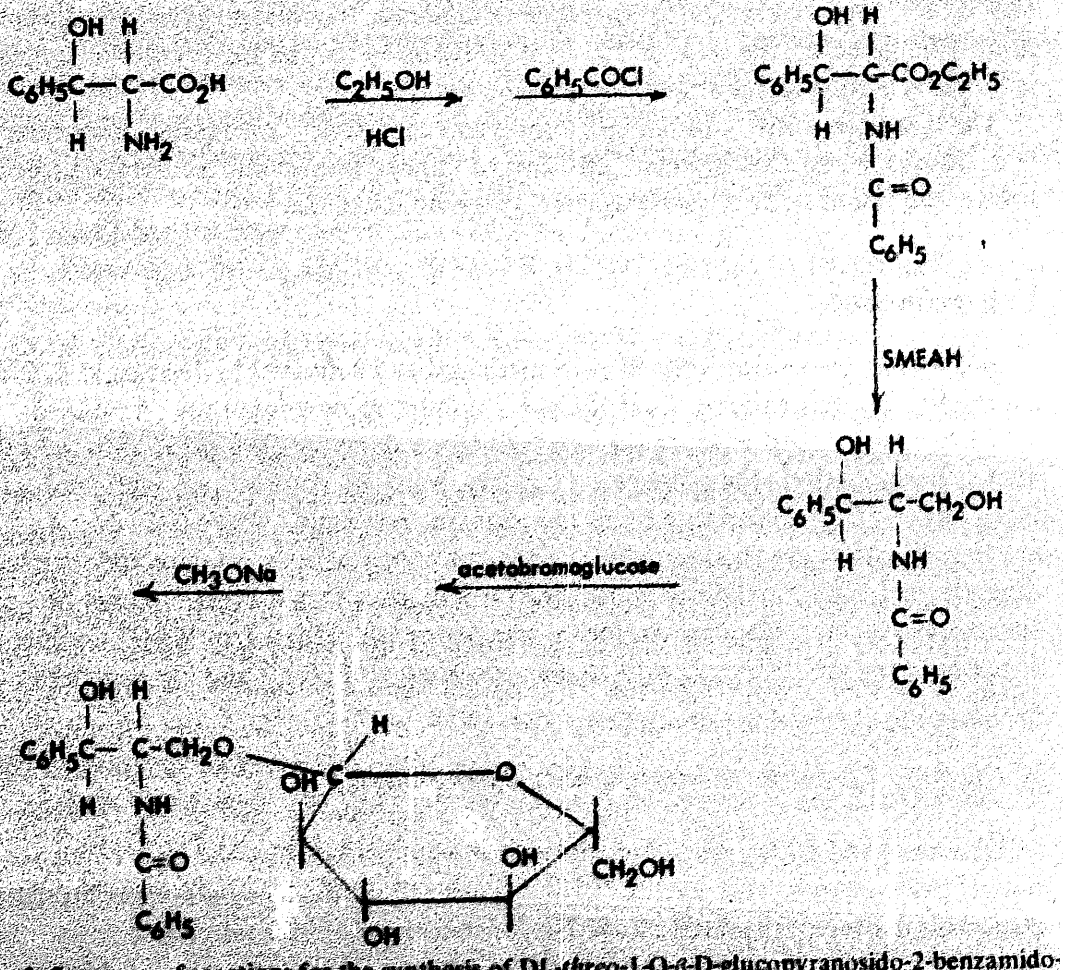

Fig. 2. Sequence of reactions for the synthesis of DL-*threo*-1-O-β-D-glucopyranosido-2-benzamido-3-hydroxy-3-phenylpropane from DL-*threo*-3-phenylserine. See text for details.

correct elementary analyses. Mass spectrometry disclosed that the condensation had occurred at the primary hydroxyl group. Both from the procedural method and the infrared (i.r.) absorption at 890–900 cm^{-1} which is indicative of a β-glycosidic linkage [8], it was concluded that the compound obtained was DL-*threo*-1-*O*-β-D-glucopyranosido-2-benzamido-3-hydroxy-3-phenylpropane.

The corresponding DL-*erythro* analogue was prepared by the same sequence of reactions. Even though TLC revealed a single component and the IR spectrum showed the expected absorption bands, considerable variations in melting points were obtained. Several crystallizations were necessary, with considerable loss in yield, to obtain a product of constant metling point.

Preparation of the *O,N*-dibenzoate of DL-*threo*-3-phenylserine ethyl ester prior to treatment with SMEAH did not improve the yield of the *N*-benzoyl derivative of DL-*erythro*-3-phenyl-1,3-dihydroxy-2-aminopropane. The corresponding *N*-acetyl derivative because of its insolubility could not be efficiently reduced with SMEAH under a variety of conditions. The DL-*threo*-3-phenyl-2-benzamido-1,3-dihydroxypropane, which gave a dimesyloxy derivative in fair yield, is in agreement with the m.p. of 165–166°C reported earlier for the same compound, B series, prepared via another route [7]. However, the m.p. of 157–159°C reported for the DL-*erythro*-analogue, A series, is in disagreement with the present result.

The biological activity of the oxazolines and cerebroside analogues were tested by Drug Research and Development, Division of Cancer Treatment, National Cancer Institute and found to be negative against lymphoid leukemia.

II. Experimental

Silicic acid, previously washed with methanol and activated by heating at 120°C overnight, was packed from chloroform for column chromatography. A ratio of 10 : 1 of adsorbent to product was used. TLC was done on silica gel G with the solvent systems diethyl ether–benzene (1 : 1) (solvent A), chloroform–methanol–conc NH_4OH (65 : 35 : 5) (solvent B), or chloroform–methanol–water (65 : 25 : 4) (solvent C). After chromatography, the various components were detected with iodine vapor. IR determinations were done on KBr discs with a Perkin–Elmer 567 IR spectrophotometer. Mass spectrometry was performed with a Jeol MS-07 double focussing mass spectrometer with an electron energy of 30 eV and ion source of 170°C.

A. DL-threo-2,5-diphenyl-4-carboethoxy-2-oxazoline (I)

DL-*threo*- and DL-*erythro*-3-phenylserines were prepared by the base condensation of benzaldehyde and glycine [9] and resolved by means of *p*-dioxane into their respective diastereomers [10]. Some DL-*threo*-compound was purchased from Aldrich Chemical. The acids were converted into their ethyl esters hydrochlorides and free base ethyl esters as previously described [9].

To 10.5 g of DL-*threo*-3-phenylserine ethyl ester in 200 ml of dry chloroform was added 11.2 g of benzimidoethyl ether hydrochloride [5,11]. After refluxing for 3 hr, the reaction mixture was filtered and the filtrate was concentrated to a sirup under reduced pressure. The sirup was dissolved in 200 ml of ethyl acetate, washed several times with water, dried over Na_2SO_4, and the product, after removal of solvent, was crystallized from *n*-heptane. Yield, 9.4 g; m.p. 85–86°C. The product gave a negative ninhydrin reaction and showed a single component on TLC with solvent A. No IR absorption was seen for hydroxyl at 1000 cm^{-1} and 3200 cm^{-1} and a strong band appeared at 1640 cm^{-1} for C=N.

Analysis Calcd. for $C_{18}H_{17}NO_3$: C, 73.18; H, 5.80. Found: C, 73.33; H, 5.87.

B. DL-threo-2,5-diphenyl-4-hydroxymethyl-2-oxazoline (II)

Compound I, 6.0 g, in 50 ml of dry toluene and 100 ml of dry diethyl ether was added during approx. 20 min to a magnetically stirred solution of 16.0 ml of SMEAH (70% solution in benzene) in 100 ml of diethyl ether immersed in an ice bath. After being stirred continuously for 1 hr, the reaction mixture was poured into 80 ml of cold 1 N NaOH followed by the addition of 300 ml of ethyl acetate. The organic layer was washed several times with water and filtered. After removal of solvent, the dried white solid was crystallized from 75 ml of nitromethane. Yield, 4.3 g; m.p. 162–163°C The product was ninhydrin negative and showed one component on TLC with solvent A. There were strong IR bands for OH, 1000 cm^{-1} and 3200 cm^{-1}, and C=N, 1640 cm^{-1}, and none for ester carbonyl at 1725 cm^{-1}.

Analysis Calcd. for $C_{16}H_{15}NO_2$: C, 75.85; H, 5.97. Found: C, 75.73; H, 5.94.

C. DL-threo-2-methyl-4-hydroxymethyl-5-phenyl-2-oxazoline (III)

To 20.9 g of DL-*threo*-3-phenylserine ethyl ester in 100 ml of dry chloroform was added 16.5 g of acetimidoethyl ether hydrochloride [11]. After refluxing for 3 hr, the reaction mixture was treated in the same manner as in the preparation of compound I. The dried sirup, obtained after concentration of the washed ethyl acetate solution, in 150 ml of dry toluene was added to 34.6 ml of SMEAH under the same conditions used in the preparation of compound II. The product was crystallized successively from nitromethane and benzene. Yield, 6.8 g; m.p. 114–115°C. TLC with solvent A revealed a single component. The IR spectrum showed absorption bands for OH, 1000 cm^{-1} and 3180 cm^{-1}, and C=N, 1660 cm^{-1}, and none for ester carbonyl at 1725 cm^{-1}.

Analysis Calcd. for $C_{11}H_{13}NO_2$: C, 69.07; H, 6.85. Found: C, 69.21; H, 6.88.

D. DL-threo-N-benzoyl-3-phenylserine ethyl ester (IV)

To 24.6 g of DL-*threo*-3-phenylserine ethyl ester hydrochloride in 300 ml of 10% $NaHCO_3$ and 100 ml of ethyl acetate surrounded by an ice bath was added over a

15 min interval with magnetic stirring 20 ml of distilled benzoyl chloride in 100 ml of ethyl acetate. After 2 hr of stirring at room temperature, the water washed upper phase was concentrated and the dried residue was crystallized from toluene. Yield, 19.5 g; m.p. 90–91°C; ninhydrin reaction, negative. IR absorption bands were present for ester, 1725 cm^{-1}, amide, 1540 cm^{-1} and 1620 cm^{-1}, and secondary hydroxyl, 1050 cm^{-1}.

Analysis Calcd. for $C_{18}H_{19}NO_4$: C, 69.02; H, 6.11. Found: C, 69.17; H, 6.03.

E. DL-threo-3-phenyl-2-benzamido-1,3-dihydroxypropane (V)

Under the same conditions used for the preparation of compound II, 15.6 g of compound IV was added to 35 ml of SMEAH in 100 ml of toluene. The dried residue was crystallized from nitromethane. Yield, 8.9 g; m.p. 165–166°C. IR absorption bands were present for amide, 1530 cm^{-1} and 1620 cm^{-1}, and hydroxyl, 1000 cm^{-1} and 1055 cm^{-1}.

Analysis Calcd. for $C_{16}H_{17}NO_3$: C, 70.81; H, 6.32. Found: C, 70.48; H, 6.23.

F. DL-threo-3-phenyl-O,N-dibenzoyl serine ethyl ester (VI)

To 24.6 g of DL-*threo*-3-phenylserine ethyl ester hydrochloride in 175 ml of dry pyridine surrounded by an ice bath was added 20 ml of benzoyl chloride. After standing overnight in the refrigerator, the reaction mixture was poured into crushed ice-water. The product was removed with 300 ml of ethyl acetate. After concentration of the water washed organic layer, the dried residue was crystallized from toluene. Yield, 10.4 g; m.p. 157–158°C.

Analysis Calcd. for $C_{25}H_{23}NO_5$: C, 71.90; H, 5.56. Found: C, 71.46; H, 5.41.

G. DL-threo-3-phenyl-2-benzamido-1,3-dimesyloxypropane (VII)

To 1.35 g of compound V in 50 ml of dry pyridine surrounded by an ice bath was added 3.0 ml of mesyl chloride. After standing at room temperature for several hours, the reaction mixture was poured into crushed ice-water. The product was removed with 200 ml of ethyl acetate, washed with water, concentrated, and the dried residue was crystallized from nitromethane. Yield, 0.31 g; m.p. 159–161°C. IR bands for SO_2O were present at 1170 cm^{-1} and 1350 cm^{-1}.

Analysis Calcd. for $C_{18}H_{21}O_7NS_2$: C, 50.79; H, 4.93. Found: C, 50.15; H, 5.01.

H. DL-threo-3-phenyl-2-n-decanamido-1,3-dihydroxypropane (VIII)

n-Decanoyl chloride [12], 19.0 g, in 100 ml of ethyl acetate was added to 24.6 g of DL-*threo*-3-phenylserine ethyl ester hydrochloride in 300 ml of ethyl acetate under the same conditions used for the preparation of compound IV. After removal of the ethyl acetate, the dried residue was dissolved in 125 ml of toluene and added to 70

ml of SMEAH in 100 ml of toluene under the same conditions described for the preparation of compound II. The dried residue was crystallized from acetonitrile. Yield, 15.3 g; m.p. 96–97 °C; ninhydrin reaction, negative. TLC with solvent A showed a single component.

Analysis Calcd. for $C_{19}H_{31}NO_3$: C, 70.97; H, 9.72. Found: C, 70.90; H, 9.67.

I. DL-threo-1-0-β-D-glucopyranosido-2-benzamido-3-hydroxy-3-phenylpropane (IX)

Compound V, 2.70 g, 4.5 g of α-tetraacetyl glucose-1-bromide [13], and 2.76 g of mercuric cyanide in 50 ml of nitromethane, distilled over phosphorus pentoxide, were stirred magnetically in a water bath at 55–60°C. The reaction mixture was protected from moisture by a drying tube containing anhydrous $CaSO_4$. When the bulk of the reactants entered solution after about 30 min, stirring was continued at room temperature for an additional 2 hr. The clear colorless solution, containing a small quantity of insoluble white precipitate, was concentrated under reduced pressure to a sirup. After the addition of 300 ml of ethyl acetate followed by saturation with H_2S, the reaction mixture was centrifuged and the precipitate was washed with two 25 ml portions of methanol with centrifugation. The combined supernates were washed with three 25 ml portions of 2.5% $NaHCO_3$ and water until neutral. After filtration and drying over Na_2SO_4, the filtrate was concentrated to a sirup which was then reconcentrated several times from methanol. The sirup, which dried to a stiff foam over phosphorus pentoxide in vacuo, was dissolved in 50 ml of dry methanol containing 1.0 ml of 0.5 N sodium methoxide [8]. After 4 hr at room temperature, the solution was neutralized with Dowex 50-X2 and filtered. Silicic acid, 5.0 g, was added to the filtrate which was concentrated to dryness. The product was scraped from the flask onto a silicic acid column which was developed successively with 200 ml each of chloroform and chloroform–methanol of the following compositions, 97 : 3, 90 : 10 and 80 : 20. Unreacted compound V, the desired glycoside, and unidentified material were recovered from the respective chloroform–methanol eluates of increasing polarity. The product from the 90 : 10 fraction was crystallized from nitromethane. Yield, 1.1 g; m.p. 104–106 °C. TLC with solvent B and C disclosed one component. The IR spectrum showed absorption at 890 cm^{-1} for a β-glycosidic linkage [8]. The mass spectrum was 434(w), 384(w), 326(w), 309(m), 282(s), 272(M), 254(m), 237(s), 222(s), 194(m), 164(s), and 147(s).

Analysis Calcd. for $C_{22}H_{27}NO_8$: C, 60.93; H, 6.28. Found: C, 60.53; H, 6.17.

J. DL-threo-1-0-β-D-glucopyranosido-2-n-decanamido-3-hydroxy-3-phenylpropane (X)

Compound VIII, 3.22 g, 2.76 g of mercuric cyanide, and 4.5 g of α-tetraacetyl glucose-1-bromide in 50 ml of dry nitromethane were treated under the conditions described for the preparation of compound IX. The product obtained from the chloroform–methanol (90 : 10) eluate was crystallized from nitromethane. Yield, 0.7 g; m.p. 84–86°C. TLC with solvents B and C disclosed the presence of one component.

The IR spectrum was similar to that obtained for compound IX.

Analysis Calcd. for $C_{25}H_{41}NO_8$: C, 62.06; H, 8.55. Found: C, 62.00; H, 8.45.

K. DL-erythro-N-benzoyl-3-phenylserine ethyl ester (XI)

DL-*erythro*-3-phenylserine ethyl ester hydrochloride, 24.6 g, was benzoylated under the same conditions used for the preparation of compound IV. Yield, 23.5 g; m.p. 132–133°C; ninhydrin reaction, negative. The IR spectrum and TLC were the same as that obtained with compound IV.

Analysis Calcd. for $C_{18}H_{19}NO_4$: C, 69.02; H, 6.11. Found: C, 68.95; H, 6.20.

L. DL-erythro-3-phenyl-2-benzamido-1,3-dihydroxypropane (XII)

Compound XI, 15.6 g, was treated in the same manner as in the preparation of compound V. Yield, 10.3 g; m.p. 129–130°C. The IR spectrum and TLC were the same as that obtained for compound V.

Analysis Calcd. for $C_{16}H_{17}NO_3$: C, 70.81; H, 6.32. Found: C, 70.38; H, 6.20.

M. DL-erythro-1-0-β-D-glucopyranosido-2-benzamido-3-hydroxy-3-phenylpropane

Compound XII, 2.70 g, 4.50 g of α-tetraacetyl glucose-1-bromide, and 2.76 g of mercuric cyanide in 50 ml of dry nitromethane were treated in the same manner as was described for the preparation of compound IX. The product obtained from the chloroform–methanol (90 : 10) eluate was crystallized twice from nitromethane. Yield, 0.6 g; m.p. 202–204°C. TLC with solvents B and C disclosed one component. The IR spectrum showed absorption at 900 cm^{-1} for a β-glycosidic linkage.

Analysis Calcd. for $C_{22}H_{27}NO_8$: C, 60.93; H, 6.28. Found: 60.59; H, 6.32.

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