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200. Manki Komatsu, Tsuyoshi Tomimori, and Yukiko Makiguchi*[1]: Studies on the Constituents of *Swertia japonica*. II.*[2] Isolation and Structure of New Flavonoid, Swertiajaponin.

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1. Swertiajaponin, $C_{22}H_{22}O_{11}$, m.p. 265°(decomp.), was newly isolated from the whole herb of *Swertia japonica* Makino (Gentianaceae), and identified as 6-C-β-D-glucopyranosylluteolin-7-methylether.

2. Iso-swertiajaponin, $C_{22}H_{22}O_{11}$, m.p. 258~261° (decomp.) was obtained by the acid-treatment of swertiajaponin, and formulated as 8-C-β-D-glucopyranosylluteolin-7-methylether.

3. At the same time, it was found that they were interconvertible into each other by boiling with mineral acids, reminiscent of the interrelationship between swertisin and iso-swertisin.

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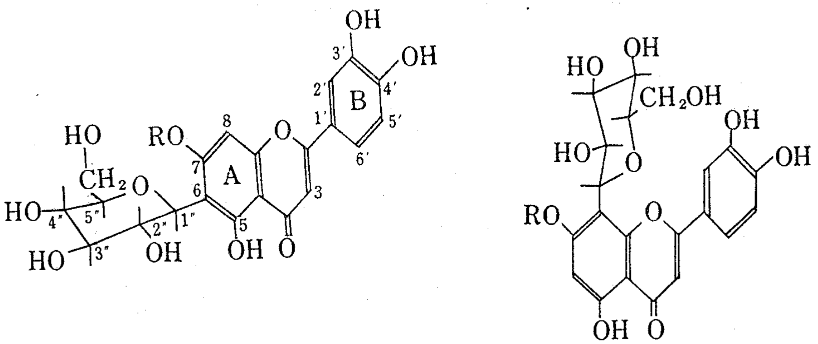
In the preceding paper,*[2] it was shown that *Swertia japonica* Makino (Japanese name "Senburi," Gentianaceae) contained three flavonoid compounds, which were isolated in pure state, *i.e.* compound (A), m.p. 243° (decomp.); compound (B), m.p. 265° (decomp.); and compound (C), m.p. 237° (decomp.).

As previously reported, compound (A) was proved to be identical with swertisin[1,2] and has been assigned to 6-C-β-D-glucopyranosylgenkwanin by the authors.

Compound (B) was a new flavonoid, which would be named swertiajaponin hereafter, and compound (C) agreed in all respects with homo-orientin (II) as described in detail in the experimental part.

The present paper is concerned with the structural elucidations of swertiajaponin and its acid-coverted isomer (named iso-swertiajaponin), which have been established as I and I′ respectively (Chart 1).



I : R=CH_3
II : R=H

I′ : R=CH_3
orientin : R=H

Chart 1.

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*[2] Part I. M. Komatsu, T. Tomimori, M. Ito: This Bulletin, 15, 263 (1967). A preliminary communication of this paper has appeared in Tetrahedron Letters, No. 15, 1611 (1966). A part of this work was reported at the Regular Meeting of Kanto Branch, Pharmaceutical Society of Japan, in Tokyo (December 1965).

1) T. Nakaoki: Yakugaku Zasshi, **47**, 144 (1927).

2) Y. Asahina, J. Asano, Y. Ueno: *Ibid.*, **62**, 22 (1942).

Swertiajaponin (I) was obtained as pale yellow powdery crystals, m.p. 265° (decomp.), and analyzed for $C_{22}H_{22}O_{11}$ having one methoxyl group. It gave a greenish brown color with ferric chloride, and the reduction tests for flavonoids were positive. It formed a hepta-O-acetate, $C_{21}H_{12}O_{10}(OCH_3)(COCH_3)_7$, m.p. 156~160°, whose infrared (IR) spectrum showed the absense of hydroxyl group. Alkali fission of I with 50% potassium hydroxide gave two products, which were proved by paper chromatography to be identical with phloroglucin monomethylether and protocatechuic acid. Boiling it with hydriodic acid in phenol gave luteolin which was further characterized as its tetra-acetate. As was expected, I was also found to be non-glycosidic as well as swertisin because of its negative Molisch's test and non-formation of sugar under the usual hydrolytic condition, and so it was suggested that I might be a C-glycosyl compound.

These data suggest that swertiajaponin possesses a 5- or 7-0-methylluteolin unit, with a $C_6H_7(OH)_4$ moiety attached to the nucleus.

Methylation of I with diazomethane yielded tri-O-methylswertiajaponin, m.p. 265~267°, which was identified as authentic tetra-O-methylhomo-orientin by mixed melting point, co-chromatography on paper, and IR spectra showing that swertiajaponin might be identical with homo-orientin-monomethylether. Further, hydrolytic decomposition of tri-O-methylswertiajaponin with boiling aqueous barium hydroxide gave 3,4-dimethoxyacetophenone and a degradation product, which was identical with 3-C-β-D-glucopyranosyl-6-hydroxy-2,4-dimethoxyacetophenone (as penta-acetate, m.p. 170°), prepared from di-O-methylswertisin.[*2]

The presence of a 7-methoxyl group in I was confirmed by the comparison of its ultraviolet (UV) spectra with those in the presence of sodium acetate (no change), and the presence of a *o*-dihydroxy group was detected by the 28 mμ bathochromic shift of the long-wave length band on the addition of a mixture of boric acid and sodium acetate (Table I).[3]

Moreover, partial methylation[4] of homo-orientin with diazomethane in the presence of chelating agent formed swertiajaponin.

From these results the structure of swertiajaponin was established as 6-C-β-D-glucopyranosylluteolin-7-methylether, which was further supported by the following nuclear magnetic resonance (NMR) data. As shown in Fig. 2, in the spectrum of hepta-O-acetyl-

Table I. Ultraviolet Absorption Spectra (λ_{max} mμ (log ε))

Solvent	I	I′	II
EtOH	259(4.30)[a]	259(4.28)[a]	259(4.32)[a]
	271(4.31)	272(4.29)	271(4.33)
	350(4.38)	351(4.35)	352(4.39)
EtOH-AlCl₃	280(4.40)	280(4.39)	278(4.43)
	304(4.10)[a]	306(4.08)[a]	304(4.08)[a]
	430(4.40)	428(4.40)	426(4.46)
EtOH-NaOAc	259(4.30)[a]	259(4.28)[a]	272(4.39)[a]
	270(4.33)	271(4.32)	279(4.40)
	360(4.18)[a]	358(4.16)[a]	327(4.15)[b]
	410(4.19)	410(4.17)	384(4.30)
EtOH-H₃BO₃-NaOAc	265(4.43)	265(4.40)	265(4.45)
	378(4.39)	380(4.37)	382(4.36)

a) shoulder *b*) barely discernible maximum in a minimum.

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3) L. Jurd: "The chemistry of Flavonoid Compounds," T.A. Geissman, Ed., 107 (1962). Pergamon Press, London.

4) M. Shimizu, M. Ota: Yakugaku Zasshi, **71**, 879 (1951).

swertiajaponin (36H), three proton signals display typical signal pattern for B-ring protons (ABX type) : H_2', doublet (J=2 c.p.s.) at 2.26τ; H_5' doublet (J=8 c.p.s.) at 2.63τ; and H_6', quartet (I_{meta}=2 c.p.s., J_{ortho}=8 c.p.s.) at 2.25τ. Two singlets at 3.11τ (1H) and 3.45τ (1H) could be assigned to the C_8 and C_3 proton respectively. A singlet at 6.00τ (3H) indicated one methoxyl group, and a total of 21 protons, observed over the range 7.56~8.21τ, are attributable to the seven acetyl groups. The signals over the range 4.0~6.0τ account for the seven protons of the glucosyl group. One of these, a doublet centred at 5.10τ is assigned to the C_1'' proton, the large coupling constant (J=10 c.p.s.) due to a *trans*-diaxial coupling with the C_2'' proton indicating the presence of β-configuration.

In addition, treatment of I with boiling 10% sulfuric acid yielded equilibrium mixture of two flavonoids, one being identical in Rf values with the original swertiajaponin, and the other was suggested to be the 8-C-isomer of I due to a Wessely-Moser rearrangement, considering the interconvertibilities between 6- and 8-C-glycosylflavonoids.*2,5~9)

TABLE II. Rf Values on Paper Chromatogram

	I	I'	II	Orientin	Products after treatment with 10% H_2SO_4		
					of I	of I'	of II
Solv. 1	0.41	0.15	0.33	0.17	0.41 0.15	0.41 0.15	0.33 0.17
Solv. 2	0.71	0.56	0.63	0.52	0.71 0.56	0.71 0.56	0.63 0.52
Solv. 3	0.45	0.34	0.45	0.31	0.45 0.34	0.45 0.34	0.45 0.31
Solv. 4	0.58	0.44	0.52	0.35	0.58 0.44	0.58 0.44	0.52 0.35

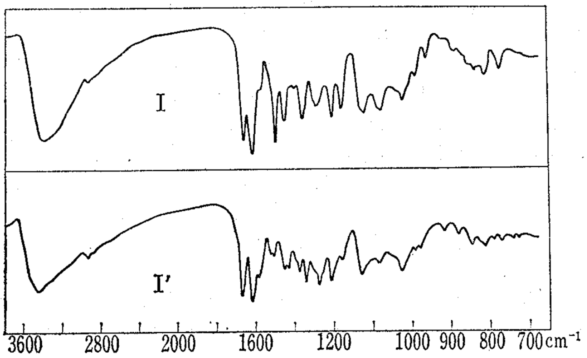

Fig. 1. Infrared Absorption Spectra of I and I' (in KBr)

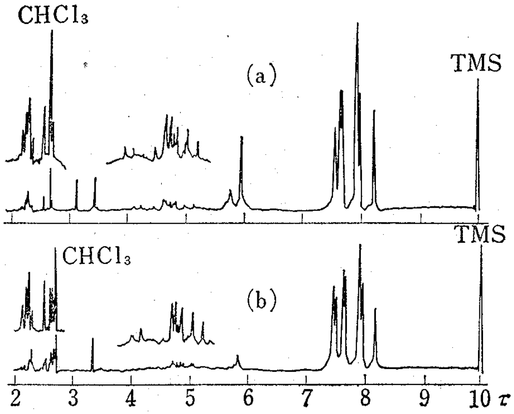

Fig. 2. Nuclear Magnetic Resonance Spectra of (a) and (b) in $CDCl_3$, measured at 60 Mc.p.s.

(a) Hepta-O-acetylswertiajaponin
(b) Octa-O-acetylhomo-orientin

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5) M.K. Seikel, T.A. Geissman : Arch. Biochem. Biophys., **71**, 17 (1957).
6) R.M. Horowitz, B. Gentili : Chem. & Ind., **1964**, 498.
7) L. Hörhammer, H. Wagner, H. Nieschlag, G. Wildi : Arch. Pharmaz. Ber. dtsch. pharmaz. Ges., **292**, 380 (1959).
8) B.H. Koeppen, D.G. Roux : Biochem. J., **83**, 507 (1962); **97**, 444 (1965).
9) This ring-isomeric change was observed in flavonoidal alkaloids, ficine and iso-ficine. S.R. Johns, J.H. Russel, M.L. Heffernan : Tetrahedron Letters, No. 24, 1987 (1965).

The isomer (I′), for which the name iso-swertiajaponin is proposed, was obtained as yellow powdery crystals, m.p. 258~261° (decomp.), $C_{22}H_{22}O_{11}$. It gave positive color reactions for flavonoid and negative Molisch reaction. It also having 7-O-methylluteolin nuclei was shown by the similarity of its UV spectra with that of swertiajaponin and the shifts in the spectra obtained with various diagnostic reagents (Table I). It was also supported by the facts that I and I′ were interconvertible into each other after treatment with mineral acid, and alkali fission of I′ afforded phloroglucin monomethylether and protocatechuic acid.

On the basis of these date, it seemed reasonable to assume that iso-swertiajaponin was identical with 8-C-β-D-glucopyranosylluteolin-7-methylether.

Thus, it has now become evident that flavonoid compounds in the whole herb of *Swertia japonica* MAKINO consist of three flavon C-glycoside, *i.e.* swertisin, swertiajaponin, and homo-orientin. Although iso-swertisin and iso-swertiajaponin have not yet been found in nature, they will be found in time, considering the some examples of which corresponding 6- and 8-C-glycosylflavonoids[*2,5,8)] are naturally occurring.

Experimental

All melting points were uncorrected. UV spectra were measured after Jurd,[10,11)] using a Hitachi Recording Spectrophotometer EPS-2U type. IR spectra were recorded on a JASCO DS-301 Spectrophotometer. MNR spectra were determined at 60 Mc. in $CDCl_3$ solutions containing TMS as an internal standard, using a JNM C-60 Spectrophotometer. Molecular weights were determined, using a Hitachi Molecular Weight Apparatus Model 115 (based on Hill's original principles). Paper chromatography was carried out by the ascending method, using Toyo Filter Paper No. 50 and solvent systems of (1) 15% AcOH (solv. 1), (2) 60% AcOH (solv. 2), (3) BuOH-AcOH-H_2O (4:1:5 by volume) (solv. 3), and (4) AcOEt-HCO_2H-H_2O) (10:2:3 by volume) (solv. 4).

Isolation of Flavonoids——The flavonoids (25 g.) obtained from the whole herb of *Swertia japonica* (6 kg.) as described in the preceding paper,[*2] was dissolved in MeOH, and chromatographed on a column of Polyamide Woelm (2 kg.), using MeOH as an eluant. The eluate was collected in 25 ml. fractions, giving fractions 1 to 230. Fractions 1~50 afforded swertisin, m.p. 243° (decomp.). Fractions 83~135 indicating only one spot on paper chromatogram of the flavonoid were combined and evaporated to dryness, giving crude swertiajaponin, Yield 3 g. Fractions 160~230 were combined and evaporated to dryness, giving crude homo-orientin. Yield 3 g.

Swertiajaponin (I)——Crude swertiajaponin was recrystallized from 50% EtOH as pale yellow powdery crystals, m.p. 265° (decomp.). Color reactions: $FeCl_3$(+), Mg-HCl(+), Zn-HCl(+), Zircon-citric acid(−), Molisch(−), Gibbs(+). $[\alpha]^{20}_{D}$ −2.6° (c, 0.5, pyridine). PPC: (Table II). TLC: Rf 0.36 (AcOEt-MeCOEt-HCO_2H-H_2O=5:3:1:1) on Silica gel G), 0.36 (15% AcOH on Avicel SF or cellulose powder), 0.65 (MeOH on Polyamide Woelm for TLC). *Anal.* Calcd. for $C_{22}H_{22}O_{11}$: C, 57.14; H, 4.80. Found: C, 56.65; H, 5.09.

Hepta-O-acetylswertiajaponin——I (0.2 g.) was acetylated with Ac_2O and pyridine (3 hr. at 110°). The mixture was poured into ice water and allowed to harden. The amorphous acetate was recrystallized from $CHCl_3$-hexane to colorless microcrystalline solid, m.p. 156~160°. Mg-HCl(+), Zn-HCl(+), $FeCl_3$(−). *Anal.* Calcd. for $C_{36}H_{36}O_{18}$: C, 57.14; H, 4.80; OMe, 4.10; mol. wt., 756.65. Found: C, 56.88; H, 5.17; OMe, 3.60; mol. wt., 769 (osmometric, solv., $CHCl_3$).

Deacetylation of Hepta-O-acetylswertiajaponin——The acetate (0.1 g.) was deacetylated by suspending it in saturated $Ba(OH)_2$ solution (40 ml.). The mixture was allowed to stand overnight and acidified with dil. HCl to pH 5, giving a yellow solution, which was passed through a Polyamide column (5 g.), and then washed with water. The absorbed matter was eluted with MeOH. Removal of the solvent afforded swertiajaponin in pale yellow powdery crystals, m.p. and mixed m.p. 265° (decomp.). Acetylation of this compound by Ac_2O-pyridine regenerated the heptaacetate, m.p. and mixed m.p. 156~160°.

Tri-O-methylswertiajaponin——A dry ethereal solution of CH_2N_2 generated from nitrosomethylurethane (5 ml.) was added to a MeOH solution of I (0.1 g.). The mixture was maintained at 5° for 24 hr. with occasional shaking and finally at room temperature for a further 24 hr. After removal of the solvent, the residue was repeatedly methylated by the same method.

The crude product was washed with ether, and recrystallized from MeOH with activated charcoal, forming colorless needles, with a negative ferric reaction, m.p. 265~267°, which was identified as tetra-O-methyl-

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10) L. Jurd, R.M. Horowitz: J. Org. Chem., **22**, 1618 (1957).
11) L. Jurd: Arch. Biochem. Biophys., **63**, 376 (1956).

homoorientin, prepared from authentic homo-orientin, by the comparison of IR spectra and mixed m.p. UV $\lambda_{\max}^{\mathrm{EtOH}}$ mμ (log ε) : 245 (4.27), 336 (4.33). PPC (solv. 2) : Rf 0.87 (blue fluorescence).

Alkali Fission of I——A mixture of I (10 mg.) and 50% KOH solution (3 ml.) was refluxed in an atmosphere of N_2 for 30 min. The reaction mixture was diluted with H_2O, acidified with dil. H_2SO_4 and extracted with ether. The ether extract was subjected to paper chromatography using diazotized sulfanilic acid as a spray reagent. Two spots were revealed on the paper chromatograms of the two phenolic substances, the Rf value of which agree with those of phloroglucin monomethylether[12] and protocatechuic acid respectively.

Decomposition of I with Hydriodic Acid——A mixture of I (0.5 g.), PhOH (10 ml.), and HI (12 ml., $d=1.7$) was gently boiled under reflux for 7.5 hr. After being cooled, the reaction mixture was poured into 1% $NaHSO_3$ solution (100 ml.). A yellow deposit was obtained which on crystallization from 50% EtOH gave yellow needls, m.p. 325°(decomp.). Admixture with authentic luteolin did not depress the melting point and the IR spectrum was also found to be superimposable with that of luteolin. PPC : Rf 0.05 (solv. 1), 0.48 (solv. 2), 0.79 (solv. 3), 0.88 (solv. 4). Its acetate, prepared by the Ac_2O-pyridine method, melted at 224°.

Hydrolytic Fission of Tri-O-methylswertiajaponin with $Ba(OH)_2$——Tri-O-methylswertiajaponin (0.1 g.) was boilded under reflux with saturated $Ba(OH)_2$ solution (20 ml.) in an atmosphere of N_2 for 2 hr. The reaction mixture was cooled, extracted with ether. After removal of ether, the residue was identified as 3,4-dimethoxyacetophenone (as its semicarbazone, m.p. 218°). The homogeneous alkaline solution was acidified with dil. H_2SO_4 to pH 6, and evaporated to dryness. The solid residue was extracted with boiling ecetone. Evaporation of the solvent left a white amorphous powder. Acetylated with Ac_2O-pyridine, it gave an acetate which were recrystallized from ether in colorless prisms, m.p. 170°. The acetate was identical with 3-C-β-D-glucopyranosyl-6-hydroxy-2,4-dimethoxyacetophenone-penta-acetate*[2] by the comparison of mixed m.p., and NMR spectra. *Anal.* Calcd. for $C_{14}H_{10}O_7(OCH_3)_2(COCH_3)_5$: C, 54.93; H, 5.67; OMe, 10.92; mol. wt., 568.52. Found : C, 54.90; H, 5.80; OMe, 10.96; mol. wt., 570.0 (osmometric, solv., $CHCl_3$).

Partial Methylation of Homo-orientin[4]——A dry ethereal solution of CH_2N_2 generated from nitrosomethylurethane (3 ml.) was added to a MeOH solution of homo-orientin (466 mg. or $1\times10^{-3}M$), NaOH (80 mg. or $2\times10^{-3}M$), and H_3BO_3 (124 mg. or $2\times10^{-3}M$). The mixture was allowed to stand overnight at 5°.

After removal of the ether, H_2O (200 ml.) was added, acidified with dil. HCl, and distilled off MeOH. The acidic aqueous solution was passed through a column of Polyamide (10 g.), and then washed with H_2O. The absorbed substance was eluted with MeOH. Removal of the solvent from the eluate afforded crude product which was revealed on paper chromatograms of a mixture of methylated homo-orientin. PPC (solv. 2) : Rf 0.72 (main), 0.79 (trace), 0.87 (trace, blue fluorescence) : cf. swertiajaponin (Rf 0.72), tetra-O-methylhomo-orientin (Rf 0.87, blue fluorescence). After liquid chromatography on a column of Polyamide, using MeOH-acetone (4:1) as an eluant, the main product was obtained as pale yellow powdery crystals. m.p. 265° (decomp.) underpressed on admixture with swertiajaponin. Yield 50 mg.

Conversion of I to Iso-swertiajaponin (I′)——A mixture of I (0.9 g.) and 10% H_2SO_4 solution (500 ml.) was refluxed for 8 hr. After being cooled, the yellow solution was neutralized with saturated $Ba(OH)_2$ Solution, evaporated to dryness. The residue was extraced several times with boiling MeOH. The combined MeOH filtrate and washings were concentrated to small volume and applied to 25 sheets of Toyo Filter Paper No. 50 (40 cm. × 40 cm.). The chromatograms were developed in solv. 2 and two major components were well separated. The area of lower band (Rf about 0.56) was cut out, and the strips were extracted with boiling 90% MeOH.

After removal of the solvent, the yellow powder obtained was recrystallized from acetone-MeOH, giving yellow powdery crystals, m.p. 258~261°(decomp.). Yield 30 mg. It gave a greenish brown color with $FeCl_3$, an orange color with Mg-HCl, an orange color with Zn-HCl, and negative Molish reaction. TLC : Rf 0.51 (AcOEt-MeCOEt-HCO_2H-H_2O=5:3:1:1 on silica gel G), Rf 0.11 (15% AcOH on Avicel SF). *Anal.* Calcd. for $C_{22}H_{22}O_{11}$: C, 57.14; H, 4.80. Found : C, 56.80; H, 5.13. The area of higher band (Rf about 0.7) was similarly recovered and identified as unchanged I.

Interconvertion of I and I′——5 mg. each of I and I′ was respectively boiled with 10% H_2SO_4 solution (10 ml.) for 7 hr. After colling and dilution with H_2O, the reaction mixture was passed through a column of Polyamide (2 g.). The column was washed with H_2O until the eluate was neutral. No sugar was detected in the passed solution and washings. Subsequent elution of the column with MeOH afforded the mixture of flavonoid. In each case, two spots were revealed on the paper chromatograms of the two flavonoids (Table II), indicating the occurrence of I and I′ in each reaction mixture.

Alkali Fission of I′——By treating I′ (2 mg.) as described above for the alkali fission of I, the degradation product was obtained, which was identical with phloroglucin monomethylether and protocatechuic acid by paper chromatography.

Homo-orientin (II)——Crude homo-orientin was recrystallized from 50% EtOH as yellow needles, m.p. 236~237°(decomp.). Color reaction : $FeCl_3$(+), Mg-HCl (+), Zn-HCl (+), Molisch (−), Gibbs (+). Rf values (Table II) agreed with those of authentic homo-orientin. Admixture with authentic sample did not depress the melting point and IR spectrum was also found to be superimposable with that of homo-orientin. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot H_2O$: C, 54.08; H, 4.72. Found : C, 54.15; H, 4.79.

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12) J. Herzig, F. Aigner : Monatsch, **21**, 435 (1900).

Treatment of II with 10% H_2SO_4——II (10 mg.) was treated as described above for the interconvertion of I and I′. The reaction product revealed two spots on paper chromatograms of two flavonoid compounds, the Rf values (Table II) of which were found to be the same as those of authentic orientin and homo-orientin respectively.

Acetate obtained by the Ac_2O-pyridine method appeared as white powdery crystals ($CHCl_3$-hexane) having a negative ferric reaction, m.p. 137~140°. Admixture with authentic octa-O-acetylhomo-orientin did not depress the melting point. *Anal.* Calcd. for $C_{37}H_{36}O_{19}$: C, 56.63; H, 4.63. Found: C, 56.45; H, 4.63.

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