CHEMICAL MODIFICATION OF ERYTHROMYCINS
II. 8-HYDROXYERYTHROMYCIN A*

KRZYSZTOF KROWICKI and ALEKSANDER ZAMOJSKI

Institute of Organic Chemistry, Polish Academy of Sciences
Warsaw, ul. Kasprzaka 44/52, Poland

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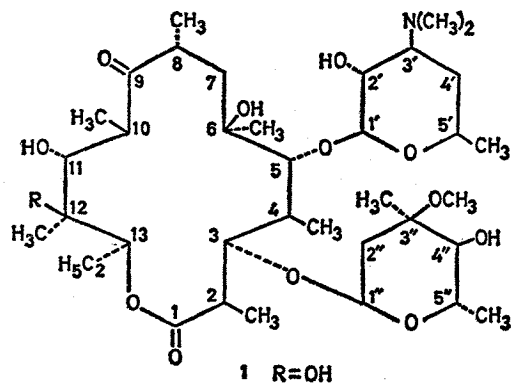
Hydroxylation of the N-oxide of erythromycin A 8,9-anhydro-6⁹-hemiketal (**2**) with *m*-chloroperbenzoic acid in methanol afforded the N-oxide of 8-hydroxyerythromycin A methyl ⁹6-ketal (**3**); reduction of the N-oxide group yielded the methyl ⁹6-ketal of 8-hydroxyerythromycin A (**4**). In slightly acid medium, compound **4** readily was hydrolysed to yield 8-hydroxyerythromycin A (**6**). The same compound **6** was obtained by hydroxylating the double bond of the 8,9-anhydro-6⁹-hemiketal of erythromycin A (**1**) with *m*-chloroperbenzoic acid in a mixture of ethyl acetate and water and reducing the N-oxide formed. The structure of the new semisynthetic antibiotic **6** was established by physicochemical methods. Compound **6** is half as active *in vitro* against *Bacillus pumilus* as the parent antibiotic, *i.e.* it assays 500 μg/mg in erythromycin A units (cylinder method). However its stability against acids is 500~600 times greater. In solutions compound **6** is in a ketone-hemiketal equilibrium.

In a previous paper[1] we have described an anhydrocompound obtained from erythromycin A and established its structure as erythromycin A 8,9-anhydro-6⁹-hemiketal (**1**). It contains the enol ether group which shows high chemical reactivity[2] and offers opportunities for chemical modification of erythromycin A. Only reactions and reagents which would not cause degradation of the macrolide system could be used and hydroxylation of the double bond with organic peracids seemed promising.

Reaction of enol ether **1** with *m*-chloroperbenzoic acid was carried out in two solvents: (a) in methanol and (b) in a mixture of ethyl acetate with water with different results**.

Formation of the N-oxide in the desosamine residue is the first step in the reaction of enol ether **1** with peracid. Therefore the N-oxide of erythromycin A 8,9-anhydro-6⁹-hemiketal[1] (**2**) was used for hydroxylation in methanol

Chart 1.



* Taken from Ph. D. thesis of K. KROWICKI submitted to the Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, in May, 1970.

** The reaction of enol ether **1** with *m*-chloroperbenzoic acid in chloroform solution will be reported in the subsequent paper of this series.

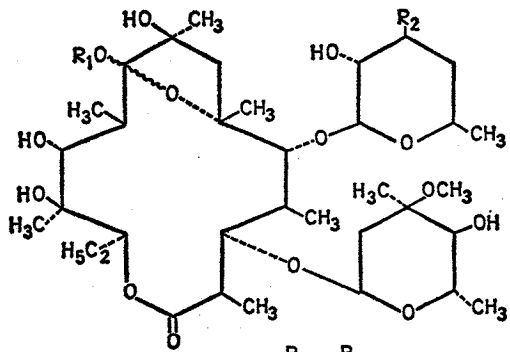
solution. A compound $C_{38}H_{69}NO_{15}$ (3) containing additional methoxy and hydroxyl groups, was the only product of this reaction. The presence of a new OCH_3 group, in addition to that occuring in the cladinose residue, was demonstrated unequivocally by the NMR spectrum. This result is consistent with expectations based on earlier evidence regarding the reaction of enol ethers with peracids. SWEET and BROWN have found[3] that treatment of 2,3-dihydrofuran with *m*-chloroperbenzoic acid in methanol yielded 2-methoxy-3-hydroxytetrahydrofuran. Consequently, we assigned the structure of 8-hydroxyerythromycin A methyl ^{9}6-ketal N-oxide to compound **3**.

Catalytic reduction of compound **3** afforded the methyl ^{9}6-ketal of 8-hydroxyerythromycin A (4). Compound **3** reacted with only one mole-equivalent of $NaIO_4$, lending additional support for the presence of a new hydroxyl group at C 8; if this group were introduced at C 10, then two molecules of $NaIO_4$ would have been used.

The reaction of enol ether 1 with *m*-chloroperbenzoic acid in an ethyl acetate-water mixture gave a single compound, $C_{37}H_{67}NO_{15}$ (5). Catalytic reduction of the N-oxide group of **5** yielded a compound $C_{37}H_{67}NO_{14}$, m.p. 143~146°C, to which we assigned the structure 8-hydroxyerythromycin A (6). The same compound was formed by mild acid hydrolysis of methylketal 4.

Hydroxylation of the double bond in **1** could, in principle, afford two stereoisomeric hydroxycompounds differing in configuration at C 8. In fact, both hydroxylations in methanol and in ethyl acetate-water yielded virtually homogeneous products, pointing to high stereoselectivity of the reactions. Taking into account the fact that the conformation of erythromycins and some of their derivatives remains stable in dilute solution with protic solvents[4], and assuming that in the same medium there are no significant changes in the spatial arrangement of the atoms in 8-hydroxyerythromycin A, we assigned to C 8 the S configuration, in view of the hypsochromic shift of the COTTON effect maximum in the CD spectrum to 284 nm from the 288 nm for erythromycin A. This shift indicated that the hydroxyl group at C 8 was situated in the same plane as the carbonyl group[5]. The intensity of the COTTON effect for both compounds in aqueous solution showed nearly identical molar amplitudes: for

Chart 2.



	R_1	R_2
3	CH_3	$NO(CH_3)_2$
4	CH_3	$N(CH_3)_2$
5	H	$NO(CH_3)_2$

Chart 3.

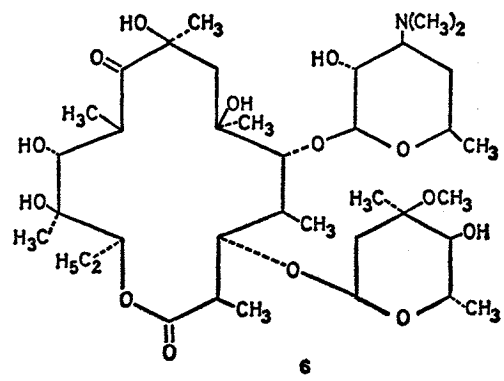


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Fig. 1. The IR spectra (KBr) of hydrorhodanides of erythromycin A (A·HSCN) and 8-hydroxyerythromycin A (AOH·HSCN).

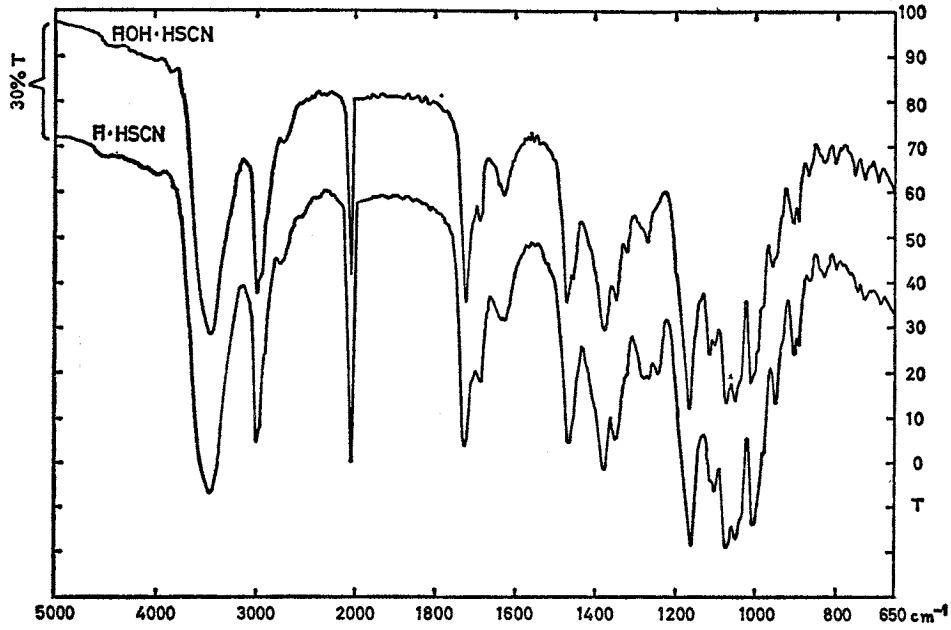
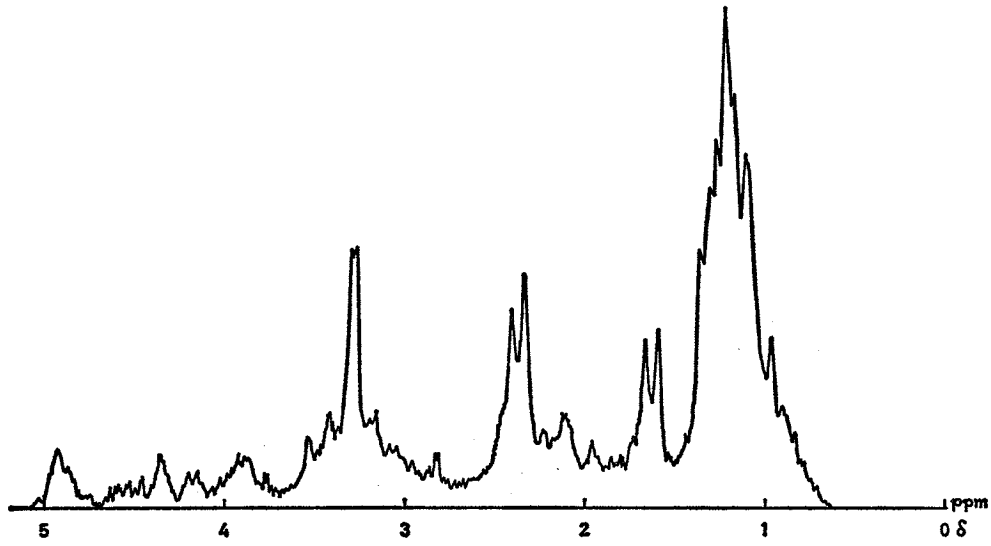


Fig. 2. The NMR spectrum of 8-hydroxyerythromycin A (6) in $CDCl_3$ (60 MHz).



erythromycin A **a** = −58, for 8-hydroxyerythromycin A (**6**) $\mathbf{a}_{100\%} = -54$*. This is expected, since the hydroxyl group in this position exerts virtually no influence on the amplitude.

The physical and chemical properties of 8-hydroxyerythromycin A (**6**) are close to those of erythromycin A. Acetylation with an equivalent amount of acetic anhydride in

* **a** 100 % represents the value calculated on 100 % of the ketone form of **6**; amounts of both ketone and hemiketal forms were evaluated from the intensity of CH_3-C 8 peaks in the NMR spectrum of **6** in D_2O solution.

Fig. 3. Hemiketal-hydroxyketone equilibrium of 8-hydroxyerythromycin A (6) in solutions.

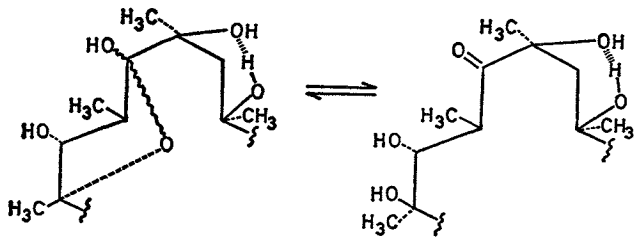
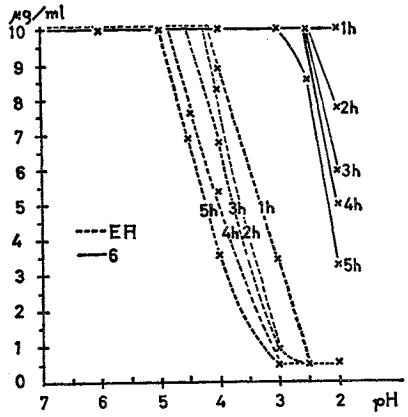


Table 1. The antibacterial spectra (*in vitro*) of erythromycin A (EA) and 8-hydroxyerythromycin A (6).

Strain	Minimum inhibitory concentration (μg/ml)	
	EA	6
Staphylococcus aureus FDA 209 P	1.95	3.9
〃 〃 111	<1.95	<1.95
〃 〃 penicillin resist.	<1.95	<1.95
Enterococcus 93	0.97	0.97
Escherichia coli 466	125	250
〃 〃 $O_{55}B_6$	125	250
〃 〃 866	125	250
Proteus OX_{22}	500	500
Bacillus subtilis 729	<1.95	<1.95
Klebsiella pneumoniae 559	125	250
Salmonella paratyphi A 192	125	250
〃 〃 B 217	31.2	125
〃 〃 C	31.2	31.2
Sarcina lutea	<1.95	<1.95
Bacillus cereus ATCC	3.9	<1.95
Shigella shigae	62.5	62.5

Fig. 4. Stability of 8-hydroxyerythromycin A (6) and erythromycin A (EA) as functions of time and pH of solution (at room temperature) in tests of activity (*in vitro*) against *Bacillus pumilus*.



pyridine affords the 2′-monoacetate; this reagent brought in excess gives the 2′,4″,11-triacetate. Compound **6** forms a hydrorhodanide; the IR spectrum of the latter closely resembles that of the hydrorhodanide of the parent macrolide (Fig. 1).

In solution compound **6**, as well as its esters, exists in equilibrium with the hemiketal form. This is indicated by the NMR spectrum in which two singlets (together of 3 H intensity) occur at δ 1.59 and 1.67; they correspond to the CH_3-C 8 of the hemiketal and ketone forms (Fig. 2). The equilibrium is also apparent from the UV spectra which show low extinctions for the ketone group maxima at 280 nm, namely 26 and 15 for aqueous and methanolic solutions, respectively; after acidification the values for both solutions increase to 35, indicating a shift of the equilibrium towards the hydroxyketone form.

Stability in acid medium is the most important feature of 8-hydroxyerythromycin A (6). As opposed to erythromycin A which even at pH 4 readily forms anhydroerythromycin A[6] containing the spiroketal structure, compound **6** remains unchanged for several hours even at pH 2.5. We assume that the enhanced stability of compound **6** to acids is due to an internal hydrogen bond between the hydroxyl groups at C 6 and C 8. Participation by the OH group at C 6 in the hydrogen bond interferes with formation of a 6^9-hemiketal which is the first stage in forming spiroketal. Dehydration of compound **6** is possible only after removal of the internal hydrogen bond in a more acidic medium. Therefore we think that it is the 12^9-hemiketal which is in equilibrium with the hydroxyketone **6** (Fig. 3).

The stabilities of 8-hydroxyerythromycin A and erythromycin A towards acids

are compared in Fig. 4., using *in vitro* activity against *Bacillus pumilus* as the test assay.

The antibacterial activity of 8-hydroxyerythromycin A *in vitro* against *Bacillus pumilus* is a half that of parent antibiotic, *viz.* 500 μg/mg (cylinder method). Its antibacterial spectrum is shown in Table 1.

8-Hydroxyerythromycin A (**6**) and erythromycin A closely resemble each other in serum curves illustrating their levels attained in rabbits, as well as in their persistence times, which were 7 hours after oral administration of a dose of 150 mg/kg of body weight. In treatment of staphylococcal infection, *Staphylococcus aureus* 551) in mice, the ED_{50} for compound **6** ranged from 150~300 mg/kg, whereas the ED_{50} for erythromycin A was less than 150 mg/kg*.

Experimental

The procedure for obtaining the 8,9-anhydro-6^9-hemiketal of erythromycin A, the apparatus used in the spectral studies and gels used in TLC and column chromatography have been described in Part I[1].

1. N-Oxide of 8-hydroxyerythromycin A methyl ^{9}6-ketal (3).

Compound **2** (1.02 g) and 0.313 g of 77 % *m*-chloroperbenzoic acid were dissolved in methanol (30 ml) and left for 4 hours. After evaporation the resisdue was treated with aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The extracts were evaporated and crystallization from acetone gave 640 mg (64.5 %) of compound **3**, m.p. 176~178°C. IR ($CHCl_3$): 3600 (OH), 1718 cm^{-1} (CO of lactone). NMR : 1.54 (s, 3 H)-CH_3 at C 8 ; 3.21 (s, 6 H)-$NO(CH_3)_2$; 3.30 (s, 3 H) and 3.44 (s, 3 H)-2 CH_3O.

Anal. Calcd. for $C_{38}H_{69}NO_{15}$ (779.94): C 58.52, H 8.92 %
Found : C 58.41, H 9.17 %

TLC[7]: ethanol-methylene chloride-ethyl ether 5 : 63 : 32, Rf 0.4. One mole of compound 3 used up 1 mole of $NaIO_4$ during 1 hour[8].

2. Methyl ^{9}6-ketal of 8-hydroxyerythromycin A (4).

Compound **3** (630 mg) dissolved in methanol was reduced with H_2/Pt. After evaporation of solvent, the residue was crystallized from ligroin, b.p. 80~100°C, affording 502 mg (80 %) of compound **4**, m.p. 124~126°C. IR ($CHCl_3$): 3530 (OH), 1703 cm^{-1} (CO of lactone). NMR : 1.52 (s, 3 H)-CH_3 at C 8; 2.26 (s, 6 H)-$N(CH_3)_2$; 3.26 (s, 3 H) and 3.40 (s, 3 H)-2 CH_3O.

Anal. Calcd. for $C_{38}H_{69}NO_{14}$ (763.94): C 59.74, H 9.11 %
Found : C 59.94, H 9.10 %

TLC[7]: ethanol-methylene chloride-ethyl ether 5 : 55 : 40, Rf 0.8.

3. N-Oxide of 8-hydroxyerythromycin A (5).

Compound **1** (35.79 g) was dissolved in a mixture of ethyl acetate (200 ml) and water (30 ml); 22.34 g of 77 % *m*-chloroperbenzoic acid were gradually added with vigorous stirring. After 4 hours the mixture was shaken with aqueous Na_2CO_3. The aqueous layer was extracted several times with ethyl acetate. The combined ethyl acetate layers gave, after evaporation and crystallization from acetone, 30.6 g (80 %) of compound **5**, m.p. 215~221°C. NMR : 1.57 (s, 3 H) CH_3 at C 8; 3.26 (s, 6 H)-$NO(CH_3)_2$; 3.38 (s, 3 H)-CH_3O.

4. 8-Hydroxyerythromycin A hydrorhodanide.

(**1**) Compound **5** (30.6 g) was reduced with H_2/Pt in methanol. After evaporation of the solvent, the residue, dissolved in some water, was made neutral with aqueous acetic acid. Upon addition of aqueous potassium rhodanate, 30 g (91 %) of 8-hydroxyerythromycin A hydrorhodanide precipitated, m.p. 184~186°C (ethyl acetate). IR (KBr) is shown

* *In vitro* microbiological tests were performed by Mrs M. Zajaczkowska, from this Institute. *In vivo* tests were carried out in the Institute of Antibiotics, Warsaw, under supervision of Dr. W. Chojnowski.

in Fig. 1.: 3500 (OH), 2080 (SCN), 1735 (CO of lactone), 1695 cm^{-1} (CO of ketone). UV: λ_{max} 281 nm, ε 24 (methanol).

Anal. Calcd. for $C_{37}H_{67}NO_{14}\cdot HSCN$ (809.0): C 55.18, H 8.52, N 3.38 %
Found: C 55.49, H 8.60, N 3.20 %

(2) An aqueous solution of compound **4** (382 mg) was adjusted to pH 6 with acetic acid. After addition of aqueous KSCN, 370 mg (90 %) of 8-hydroxyerythromycin A hydrorhodanide precipitated.

5. 8-Hydroxyerythromycin A (**6**).

8-Hydroxyerythromycin A hydrorhodanide (30 g) suspended in water was made basic with aqueous ammonia and extracted with CH_2Cl_2. The extracts gave 26 g (95 %) of compound **6**. That compound dissolved in some water, after 1 day, precipitated in its crystalline form of water solubility 4.3 mg/ml at 19°C, m.p. 143~146°C (after drying). IR (KBr): 3520 (OH), 1740 (CO of lactone), 1695 cm^{-1} (CO of ketone). UV: λ_{max} 279 nm, ε 15.4 (methanol); λ_{max} 280 nm, ε 25.5 (water); λ_{max} 275 nm, ε 35 (water, after acidification with HCl). CD: **a** −36, 284 nm (water); **a** −21, 284 nm (methanol). NMR: 1.59 and 1.67 (altogether 3 H) $-CH_3$ at C 8; 2.33 and 2.40 (altogether 6 H)-$N(CH_3)_2$; 3.27 and 3.29 (altogether 3 H)-CH_3O (Fig. 2). $[\alpha]_D^{20}$ −54.5±1° (*c* 1, methanol). pKa 8.8 (66 % DMF); pKa 9.2 (water).

Anal. Calcd. for $C_{37}H_{67}NO_{14}$ (749.91): C 59.25, H 9.01 %
Found: C 59.18, H 9.12 %

6. Stability tests of 8-hydroxyerythromycin A (**6**) and erythromycin A as a function of pH of the solution.

Erythromycin A (125,000 μg) was dissolved in 5 ml of methanol and diluted with water to 50 ml. Subsequently, 2 ml aliquots were transferred to 10 ml volumetric flasks and made up to volume with buffer of given pH. After 1,2,3,4 and 5 hours 1 ml aliquots were transferred to 50 ml volumetric flasks and made up to volume with buffer of pH 8. The final solution ought to contain, without activity loss, 10 μg/ml. By an analogous procedure 125,000 μg of 8-hydroxyerythromycin A (**6**) were tested. The activity of the samples was tested by the cylinder method against *B. pumilus* using a standard erythromycin A solution (10 μg/ml) as reference. The results are shown in Fig. 4.

7. 2'-Acetate of 8-hydroxyerythromycin A.

Acetic anhydride (0.2 ml) was added to a pyridine (5 ml) solution of compound **6** (1.5 g). After 1 day at room temperature and evaporation (under reduced pressure), the residue, dissolved in some acetone, was poured into aqueous $NaHCO_3$ and extracted with CH_2Cl_2. Purification was performed by column chromatography on 90 g of Kieselgel which contained 10 % basic Al_2O_3 (Woelm), using ethyl acetate followed by acetone as eluents. One g (63 %) of 2'-acetate, m.p. 134~137°C, was obtained. IR (KBr): 3540 (OH), 1745 (CO of lactone, ketone and acetate), 1240 cm^{-1} (CH_3COO). UV: λ_{max} 280 nm, ε 21 (methanol). NMR: 1.58 and 1.63 (altogether 3 H)-CH_3 at C 8; 2.03 (s, 3 H)-CH_3COO; 2.26 (s, 6 H)-$N(CH_3)_2$; 3.28 and 3.32 (altogether 3 H)-CH_3O. $[\alpha]_D^{20}$ −54.4±1° (*c* 1, methanol). pKa 7.6 (water). Antibacterial activity against *B. pumilus* was 490 μg/ml (cylinder-plate method).

8. 2',4'',11-Triacetate of 8-hydroxyerythromycin A.

Compound **6** (0.5 g) was acetylated in a similar manner to that described in Part I, Paragraph 2. (1)[1]. After crystallization of the crude product from ligroin, b.p. 40~60°C, 0.53 g (90%) of triacetate, m.p. 122~125°C, was obtained. IR ($CHCl_3$): 3500 (OH), 1735 (CO of lactone, ketone and acetate), 1240 cm^{-1} (CH_3COO). UV: λ_{max} 285 nm, ε 16 (methanol) NMR: 1.44 (s, 3 H)-CH_3 at C 8; 2.01 (s, 3 H)-CH_3COO; 2.07 (s, 6 H)-2 CH_3COO; 2.36 (s, 6 H)-$N(CH_3)_2$; 3.34 (s, 3 H)-CH_3O.

Anal. Calcd. for $C_{43}H_{73}NO_{17}$ (876.02): C 58.95, H 8.40 %
Found: C 58.86, H 8.57 %

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