CHEMICAL AND X-RAY STRUCTURAL INVESTIGATION OF A NEW DITERPENE LACTONE BETOLIDE

V. V. Tkachev, G. K. Nikonov,
L. O. Atovmyan, A. Ya. Kobzar',
and T. V. Zinchenko

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A crystalline substance with the composition $C_{20}H_{24}O_5$ has been isolated from the roots of common betony. The spatial structure of this new diterpene lactone, betolide, has been established from the results of UV, IR, NMR, and ^{13}C NMR spectral and x-ray structural investigations — 13-formyl-14-hydroxy-(2'-hydroxy-5'-oxotetrahydrofuro)[3',4':11,12']-$\Delta^{8,10,12}$-podocarpane, the first representative of new tetracyclic diterpenes the lactone ring of which is formed with the participation of a hydroxyl of a geminal diol. The stability of a lactone ring of this type and a high reactivity of the semiacetal hydroxyl have been shown.

From the roots of Betonica officinalis L. (Stachys officinalis), (commonbetony) growing in the Kiev Oblast we have isolated a crystalline substance with the composition $C_{20}H_{24}O_5$, mp 222-224°C, $[\alpha]_D^{20}$ + 174 ± 5° (c 1.58; acetone), M^+ 344, readily soluble in organic solvents and sodium bicarbonate solution but insoluble in water. Absorption maxima in its UV spectrum, λ_{max}, 228, 284, 347 nm (log ε 4.61, 4.24, 3.96) showed the presence in the molecule of an aromatic nucleus and of conjugated double bonds; a hypsochromic shift of the long-wave maximum in the presence of alkali, $\Delta\lambda = 40$ nm, showed the fact that it was an acid; and a bathochromic shift $\Delta\lambda = 40$ nm, in the presence of zirconyl nitrate indicated the presence of a phenolic hydroxyl hydrogen bonded to a carbonyl group.

In the IR spectrum, absorption bands were observed with ν_{max} (cm^{-1}) 3500-3350 (hydroxyl); 2980, 2920, 2880 (C-methyl and methylene groups); 1750-1770 (carbonyl of a lactone or of an acid); 1660 (carbonyl of an unsaturated ketone or of an aldehyde); and 1620, 1570 (aromatic nucleus); and also at 1480, 1360, 1315, 1180, 1130, and 1040.

The presence of hydroxy groups was confirmed by the formation of mono- and diacetates, and that of a carboxy group by the formation of a methyl ester. It was not posible to obtain a 2,4-dinitrophenylhydrazone or an oxime. On titration in ethanol, the substance added an equimolar amount of alkali. It follows from these facts that the substance isolated was an aromatic hydroxy acid or hydroxy lactone.

The PMR spectrum contained three three-proton singlets at 0.89, 0.94, and 1.20 ppm (tertiary C-methyl groups), singlets at 5.27 ppm (olefinic proton) 12.22 ppm (phenolic hydroxyl), and 10.74 ppm (proton of an aldehyde or carboxy group); and a multiplet at 2.84 ppm (methylene protons at a benzyl carbon atom). The ^{13}C NMR spectrum showed the signals of the carbon atoms of a benzene ring (161.5, 155.3, 136.64, 133.4, 124.5, and 112.18 ppm) of a carbonyl carbon (194.3 ppm), of a carbon atom linked to two oxygen atoms (98.4 ppm), and of sp^3-hybridized carbon atoms (49.1, 40.4, 35.68, 33.11, 32.6, 24.4, 20.99, 18.3, and 16.9 ppm).

Crystallization from acetone gave transparent light yellow single crystals having the shape of parallelepipeds deformed along a body diagonal. The structure of the molecule and a projection on the plane of least overlapping obtained in accordance with [1] is shown in Fig. 1.

The C9-C14 benzene ring (mean square plane $3.36x + 8.67y + 3.97z = 11.58$; maximum departure 0.02 Å) is located in the center of the molecule, forming in combination with the two C1-C10 cyclohexane rings the expected diterpene fragment. The O25H25 and H17C7O24 group-

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Institute of Chemical Sciences, Kazakhstan SSR Academy of Sciences, Alma-Ata. Division of the Institute of Chemical Physics, USSR Academy of Sciences, Chernogolovka. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 811-817, November-December, 1987. Original article submitted May 4, 1987.

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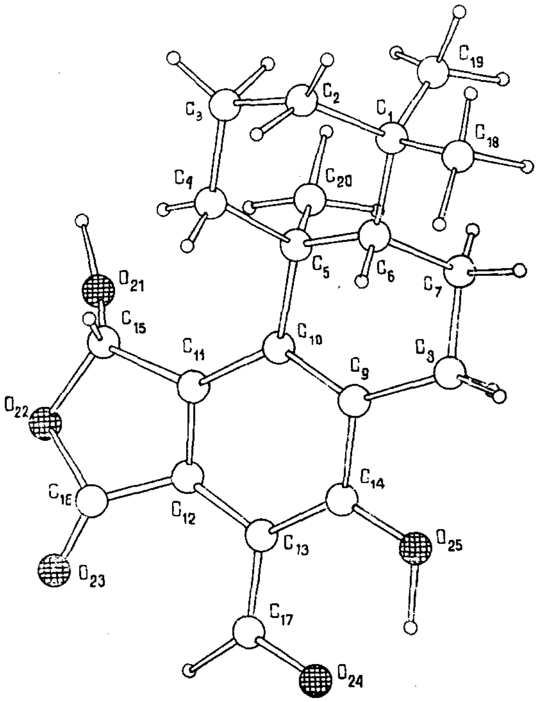
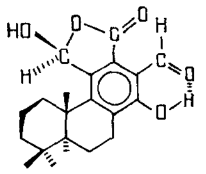


Fig. 1. Structure of the betolide molecule in a projection on the plane of least overlapping. All the distances given [sic] have an error of 0.01 Å.

ings attached to the other two atoms of the benzene ring are located in close proximity and are so oriented in space that an intermolecular hydrogen bond, O24...H25 = 1.64 Å, is realized. The other two carbon atoms of the benzene ring form part of the γ-lactone fragment unexpectedly detected. Thus, the molecule consists of a condensed system of four rings. It is striking that the C5, O22, and O25 atoms lie in the above mentioned plane of the benzene fragment with an accuracy of 0.02 Å, the C17, O24, and H17 atoms depart from it by not more than 0.07 Å, and the C8, C15, C16, and H25 atoms by not more than 0.11 Å, i.e., the majority of the atoms of the four rings are located close to the plane of the benzene ring. This can be observed clearly by considering a projection of the molecule along a straight line passing through the C8 and C10 atoms. The O21 and C20 atoms are located on one side of this plane and the atoms of the last cyclohexone ring on the other side. It is possible that the reason for this planar structure of the central part of the molecule is conjugation between the π-orbitals of the benzene ring and the C16-O23 and C17-O24 double bonds.

The C_5-C_{10} cyclohexane ring has the envelope conformation with a 122° bend about the C_5C_7 line. The second, more remote, cyclohexane ring has the chair conformation. The plane of the $C_1C_3C_4C_6$ atoms makes an angle of 167° with the main plane of the molecule. The planes of the $C_1C_2C_3$ and $C_4C_5C_6$ atoms are parallel to an accuracy of 7° and make angles of 131 and 138°, respectively, with the plane of the $C_1C_3C_4C_6$ atoms.

Thus, the substance under investigation had the following structure:



There is no information on dehydrated abietanes containing lactone rings in the Cambridge bank of organic structures containing those published up to December, 1984, and this is the first time this substance, which we have called betolide, has been described. Betolide is characterized by an originality of structure — its skeleton does not conform to the isoprene rule and the lactone ring is formed through a geminal diol (acetal), i.e., it is a representative of a new group of diterpenes.

TABLE 1. Parameters of the PMR Spectra of Betolide and Its Derivatives (in $CDCl_3$)

Substance	CS, ppm (δ scale)	Multiplicity	Integral intensity	Assignment
I	12,22	s	1	$Ar\,O\underline{H}$
	10,74	s	1	$Ar\,C\underline{H}O$
	6,79	s	1	$Ar\,COOC\underline{H}(OH)$
	2,75	m	2	$Ar\,\underline{CH_2}$
	1,33	s	3	$>C-\underline{CH_3}$
	0,93	s	6	$>C(\underline{CH_3})_2$
II	12,22	s	1	$Ar\,O\underline{H}$
	10 89	s	1	$Ar\,C\underline{H}O$
	6,21	s	1	$Ar\,COOC\underline{H}(OCH_3)$
	3.40	s	3	$Ar\,COOCH(O\underline{CH_3})$
	2,68	m	2	$Ar\,\underline{CH_2}$
	1,17	s	3	$>C-\underline{CH_3}$
	0,92	s	6	$>C(\underline{CH_3})_2$
III	10,87	s	1	$Ar\,C\underline{H}O$
	7,38	s	1	$Ar\,COOC\underline{H}(OH)$
	2,82	m	2	$Ar\,\underline{CH_2}$
	2,09	s	3	$Ar\,OCO\underline{CH_3}$
	1,18	s	3	$>C-\underline{CH_3}$
	0,88	s	6	$>C(\underline{CH_3})_2$
IV	10,88	s	1	$Ar\,C\underline{H}O$
	7,48	s	1	$Ar\,COOC\underline{H}(OCOCH_3)$
	2,78	m	2	$Ar\,\underline{CH_2}$
	2,42	s	3	$Ar\,COOCH(OCO\underline{CH_3})$
	2,12	s	3	$Ar\,OCO\underline{CH_3}$
	1,25	s	3	$>C-\underline{CH_3}$
	0,90	s	6	$>C(\underline{CH_3})_2$
V	5,40; 5,20	dd J = 14 Hz	2	$Ar\,\underline{CH_2}OH$
	5,40	s	1	$Ar\,COOC\underline{H}(OH)$
	2,78	m	2	$Ar\,\underline{CH_2}$
	1,02	s	3	$>C-\underline{CH_3}$
	0,90; 0,95	s; s	6	$>C(\underline{CH_3})_2$
VI	7,46		1	$Ar\,COOC\underline{H}(OCOCH_3)$
	5,40; 5,20	dd J = 14 Hz	2	$Ar\,\underline{CH_2}OH$
	2,67	m	2	$Ar\,\underline{CH_2}$
	2,25	s	3	$Ar\,COOCH(OCO\underline{CH_3})$
	1,92	s	3	$Ar\,OCO\underline{CH_3}$
	1,19	s	3	$>C-\underline{CH_3}$
	1,15; 0,85	s;s	6	$>C(\underline{CH_3})_2$

The semiacetal hydroxyl of betolide has pronounced acidic properties, and it participates in the formation of a methyl ether. This is shown by the IR spectrum of the methyl derivative in which the absorption band of a lactone ring is retained. Its acetylation under mild conditions forms a monoacetate at the phenolic hydroxyl, which was shown by the disappearance of the singlet at 12.25 ppm in the PMR spectrum.

The signal of the methine proton of the semiacetal in the PMR spectrum of the methyl ether had shifted upfield (Δ 0.58 ppm), while in the acetate it had shifted downfield (Δ 0.59 ppm). On acetylation under severe conditions the semiacetal hydroxyl was also acetylated, with the formation of a diacetate.

TABLE 2. Coordinates of the Atoms ($\times 10^4$, $\times 10^3$ for H) and Errors in Their Determiantion

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
C 1	470 (3)	10032 (7)	9657 (9)	H 2.1	47	1025	1322
C 2	257 (3)	9904 (7)	11785 (9)	H 2.2	—2	1083	1196
C 3	277 (3)	8663 (7)	12671 (8)	H 3.1	—3	825	1364
C 4	880 (3)	8139 (8)	12530 (9)	H 3.2	1	825	1135
C 5	1154 (3)	8103 (7)	10530 (9)	H 4.1	117	850	1344
C 6	1090 (3)	9385 (7)	9579 (9)	H 4.2	93	712	1292
C 7	1330 (3)	9413 (8)	7531 (8)	H 6	138	1003	1036
C 8	1972 (3)	9216 (8)	7589 (8)	H 7.1	131	1025	666
C 9	2168 (3)	8311 (6)	9171 (9)	H 7.2	97	865	644
C 10	1808 (3)	7817 (6)	10592 (9)	H 8.1	211	1001	675
C 11	2056 (3)	7014 (6)	12038 (8)	H 8.2	225	901	693
C 12	2643 (3)	6845 (6)	12019 (9)	H 15	164	700	1464
C 13	3016 (3)	7323 (6)	10605 (8)	H 17	389	655	1171
C 14	2765 (3)	8070 (6)	9178 (9)	H 18.1	65	1169	773
C 15	1797 (3)	6258 (7)	13680 (8)	H 18.2	101	1176	987
C 16	2807 (4)	6074 (7)	13712 (9)	H 18.3	17	1185	924
C 17	3631 (3)	7025 (7)	10571 (9)	H 19.1	—34	987	796
C 18	550 (3)	11408 (7)	9176 (8)	H 19.2	9	933	638
C 19	42 (3)	9476 (7)	8227 (9)	H 19.3	—8	850	841
C 20	877 (3)	7012 (6)	9281 (9)	H 20.1	92	607	996
O 21	1461 (2)	5305 (4)	13029 (8)	H 20.2	93	720	761
O 22	2309 (2)	5744 (5)	14702 (8)	H 20.3	43	729	911
O 23	3258 (2)	5738 (5)	14367 (9)	H 21	101	501	1324
O 24	3937 (2)	7497 (5)	9322 (9)	H 25	360	833	767
O 25	3084 (2)	8594 (6)	7745 (9)				

TABLE 3. Valence Angles ω (degrees) and Their Errors

Angle	ω	Angle	ω
C2 C1 C6	106,4 (6)	C8 C9 C10	123,8 (7)
C2 C1 C18	109,1 (7)	C8 C9 C14	114,6 (7)
C2 C1 C19	110,8 (6)	C10 C9 C14	121,5 (7)
C6 C1 C18	107,7 (6)	C5 C10 C9	119,9 (7)
C6 C1 C19	113,5 (6)	C5 C10 C11	122,4 (6)
C18 C1 C19	109,2 (7)	C9 C10 C11	117,7 (6)
C1 C2 C3	117,2 (7)	C10 C11 C12	118,4 (7)
C2 C3 C4	110,1 (6)	C10 C11 C15	132,2 (6)
C3 C4 C5	117,4 (6)	C12 C11 C15	109,5 (6)
C4 C5 C6	108,4 (6)	C11 C12 C13	125,1 (7)
C4 C5 C10	113,4 (6)	C11 C12 C16	109,0 (7)
C4 C5 C20	109,3 (6)	C13 C12 C16	126,0 (7)
C6 C5 C10	106,6 (6)	C12 C13 C14	115,7 (6)
C6 C5 C20	113,9 (6)	C12 C13 C17	122,5 (7)
C10 C5 C20	105,4 (6)	C14 C13 C17	121,8 (7)
C5 C6 C1	117,8 (6)	C9 C14 O13	121,5 (7)
C5 C6 C7	111,8 (6)	C9 C14 O25	117,5 (7)
C1 C6 C7	111,0 (6)	C13 C14 O25	121,0 (7)
C6 C7 C8	111,0 (6)	C11 C15 O21	113,5 (7)
C7 C8 C9	114,0 (7)	C11 C15 O22	102,9 (6)
C12 C16 O22	107,9 (7)	O21 C15 O22	109,3 (6)
C12 C16 O23	133,6 (8)	C15 O22 C16	110,5 (6)
O22 C16 O23	118,5 (7)	C13 C17 O24	119,3 (7)

On treatment with sodium tetrahydroborate, the aldehyde group was reduced with the formation of a triol $C_{20}H_{26}O_5$. In its PMR spectrum, the singlet corresponding to the aldehydic proton had disappeared and two doublets had appeared — 5.40 and 5.20 ppm, J = 14 Hz — due to methylene protons at a benzyl carbon atom of a primary alcohol. In this case, the signal of the semiacetal proton was retained, although it had shifted upfield (Δ 1.39 ppm). In the IR spectrum of the same substance the absorption band of the carbonyl of an aromatic aldehyde (1650 cm^{-1}) had disappeared, but the band of the lactone carbonyl remained. Thus, the lactone ring of the betolide is characterized by stability.

Acetylation of the reduction products formed a diacetate with the composition $C_{24}H_{30}O_7$, mp 154-156°C. As the features of its PMR spectrum showed, in this compound one acetyl group substituted the phenolic hydroxyl (singlet at 1.92 ppm) and the other the semiacetal hydroxyl (singlet at 2.25 ppm). In the latter case, the signal of the semiacetal proton underwent a considerable downfield shift (Δ 2.0 ppm).

EXPERIMENTAL

UV spectra were taken on a Specord UV-VIS spectrometer; IR spectra on a UR-20 instrument (in KBr); PMR spectra on a Tesla BS 487 (80 MHz) instrument; mass spectra on a MKh-1320 instrument; and ^{13}C NMR spectra on a Bruker WP-80 instrument (in $CHCl_3$).

X-Ray Structural Analysis. The experimental material was obtained on a RÉD diffractometer with Mo K_α radiation using a specimen of isometric shape with mean dimensions of ≈0.3 mm. The main crystallographic characteristics were: $C_{20}H_{24}O_5$; M = 344.39; a = 23.411(8), b = 10.843(3), c = 6.817(2) Å; V = 1730 Å^3; d_{calc} = 1.245 g/cm^3 for z = 4. The number of independent nonzero reflections with $I \geq 2\sigma$ recorded in the angular interval $\theta \leq 25°$ was 747. Analysis of extinctions led to the choice of the space group $P2_12_12_1$. The structure was determined by the direct method and was refined by means of a Rentgen-75 program [2]. In the best E-synthesis, 19 nonhydrogen atoms were localized (R = 0.22). The following alternation of difference syntheses and stages of refinement by the method of least squares permitted the coordinates of the hydrogen atoms to be determined objectively and the value of the R-factor to be lowered to 0.054 in an anisotropic full-matrix refinement by the method of least squares of the nonhydrogen atoms. The coordinates of the atoms are given in Table 2 and the values of the valence angles in the molecule in Table 3.

The purity of the substances was checked by TLC on Silufol plates in the BAW (4:1:2) system, the spots being revealed by their fluorescence in UV light. The results of elementary analysis agreed with the calculated figures. The betolide was isolated by extraction of the roots with ethanol with subsequent purification by column chromatography.

Determination of the Equivalent of Betolide (I). A solution of 0.0507 g of the substance in 20 ml of methanol was titrated with a 0.1 N solution of caustic potash to phenolphthalein. The amount of alkali solution consumed was 2.63 ml, which corresponds to an equivalent of 342.5 g.

The methyl derivative (II) of (I) was obtained by heating a 1% solution of (I) in methanol in the presence of concentrated sulfuric acid (10%) for 3 h. The liquid was diluted with water and extracted with ether, and the extract was dried and evaporated. Colorless crystals were obtained with mp 176-178°C (from methanol), composition $C_{21}H_{26}O_5$. IR spectrum, ν_{max}: 3350-3250, 2960, 2920, 2860, 1750-1710, 1650, 1470, 1350, 1315, 1180, 1120, 1040, 950, 810 cm^{-1}.

The monoacetate (III) of (I) was obtained by boiling (I) with acetic anhydride in the presence of fused sodium acetate for 2 h. Colorless crystals mp 158-160°C (from methanol); M^+ 386. IR spectrum, ν_{max}: 3250-3400, 2980, 2940, 2880, 1750, 1650, 1620, 1470, 1350, 1320, 1180, 1130, 1135, 950 cm^{-1}

The diacetate (IV) of (I) was obtained by the same method except that the mixture was heated for 4 h. Colorless crystals, mp 166-167°C (from methanol); M^+ 406. IR spectrum, ν_{max}: 3050, 2880, 1765-1795, 1700, 1650, 1620, 1465, 1380, 1330, 1285-1310, 1070, 1045, 1015, 950, 890 cm^{-1}.

Product (V) of the Reduction of (I). In small portions, 2 g of sodium tetrahydroborate was added to 0.35 g of (I) in 20 ml of methanol. The mixture was left for 12 h and was then acidified and treated with ether. After the ethereal extract had been evaporated, a colorless crystalline substance was obtained with mp 252°C (from methanol). UV spectrum, λ_{max}: 217, 257, 313 nm. IR spectrum, ν_{max}: 3250-3500, 2860, 2930, 1750-1760, 1470, 1420, 1370, 1350, 1300, 1180, 1040, 990 cm^{-1}.

The acetate (VI) of the reaction product was obtained by boiling (V) with acetic anhydride and sodium acetate for 2 h. Colorless crystals were obtained with mp 156-158°C (from aqueous acetone).

SUMMARY

1. The spatial structure of the new diterpene lactone betolide isolated from *Betonica officinalis* L. (*Stachys officinalis*) has been established.

2. It has been shown that betolide is a representative of a new group of dehydrated diterpenes the carbon skeleton of which does not conform to the isoprene rule; it includes a lactone ring formed with the participation of a geminal diol, and it has the structure of 13-formyl-14-hydroxy-(2'-hydroxy-5'-oxotetrahydrofuro)[3',4':11,12]-$\Delta^{8,10,12}$-podocarpane.

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TRITERPENE GLYCOSIDES OF Astragalus AND THEIR GENINS.

XXIII. CYCLOCANTHOGENIN FROM Astragalus tragacantha

Yu. M. Fadeev. M. I. Isaev,
Yu. A. Akimov, P. K. Kintya,
M. B. Gorovits, and N. K. Abubakirov

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The epigeal part of the plant Astragalus tragantha Habl. (Leguminosae) has yielded (in addition to cyclosieversigenin, cyclocyclosiversioside F, and β-sitosterol β-D-glucopyranoside) a new methylsteroid of the cycloartane series — cyclocanthogenin — the structure of which has been established on the basis of chemical transformations and spectral characteristics, and also of a chemical correlation with the structure of cycloasgenin C, as 24S-cycloartane-3β,6α,16β,24,25-pentaol.

Continuing investigations of the cycloartane triterpenoids of the plants of the genus Astragalus [1], we have begun a study of Astragalus tragacantha Habl. (Leguminosae). In a methanolic extract of the epigeal part of this plant, on TLC in various solvent systems, at least 18 compounds of sterol and triterpene nature were detected, and they have been designated in order of increasing polarity substances (1-18). From the total extractive substances compounds (1, 2, 5, 6, and 9-14) were isolated by column chromatography on silica gel. Glycosides (11) and (12) predominated in the extract. Substances (1, 2, and 11) were identified as cyclosieversigenin [2, 3], β-sitosterol β-D-glucopyranoside [4], and cyclosieversioside F [5, 6], respectively.

The acid hydrolysis of glycoside (12) gave the genin (I) (Scheme 1), which we have called cyclocanthogenin. The present paper is devoted to a proof of the structure of this compound.

The elementary composition of cyclocanthogenin, $C_{30}H_{52}O_5$, the presence in the PMR spectra of the signals of seven methyl groups and also of two one-proton doublets at 0.20 and 0.48 ppm (Table 1) showed that the new genin belonged to the cycloartane series [7]. At the same time, in the PMR spectrum of compound (III), obtained under more severe conditions and having the same elementary composition as genin (I), the signals of eight methyl groups and of one olefinic proton were observed. Consequently, substance (III) was a triterpenoid of the lanost-9(11)-ene series formed by the acid isomerization of cyclocanthogenin.

It followed from the elementary composition $C_{30}H_{52}O_5$ of triterpenoids (I) and (III) that all the oxygen atoms are represented by hydroxy groups, which meant that the side chains of these substances were acyclic.

In the PMR spectrum of substance (III) the signals of four protons located geminally to hydroxy functions were clearly traced at 3.40, 3.78, 4.32, and 4.60 ppm. In the sepctrum of genin (I), these protons resonated in the form of multiplets at 3.40-3.90 and 4.58 ppm with integral intensities of three and one proton units, respectively. The fifth hydroxy group was tertiary.

The mass spectra of triterpenoids (I) and (III) each exhibited the peak of an ion with m/z 329 formed by the cleavage of the C-17—C-20 bond and the ejection of one molecule of water from the polycyclic fragment (Scheme 2) [3]. This peak was the maximum peak in the spectrum of compound (III).

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