THE STRUCTURE OF EKATETRONE, A METABOLITE OF STRAINS OF *STREPTOMYCES AUREOFACIENS*

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The structure of ekatetrone has been determined from physico-chemical data obtained using the natural compound, its derivatives and products of degradation reactions. Ekatetrone was found to be the lactone of 1,8-dihydroxy-2-(1′-hydroxy-2′-carbamoyl)ethyl-9,10-anthraquinone-3-acetic acid (**I**). It is proposed that ekatetrone is related, biogenetically, to protetrone.

Ekatetrone is a metabolite formed by particular strains of *Streptomyces aureofaciens*; it inhibits the growth of EHRLICH ascites carcinoma *in vitro*, but is not active against bacteria and yeasts.[1] The production, isolation, purification and preliminary characterization of ekatetrone have been reported in an earlier paper.[1] A partial structure has been proposed.[2,3] This paper provides evidence for the full structure (**I**) and reviews the biogenetic relationships of ekatetrone to tetracyclines.

The Structure of Ekatetrone

Ekatetrone is a yellow, neutral, optically-active, crystalline solid, $C_{19}H_{13}NO_7$, which formed a triacetate when treated with acetic anhydride-sulphuric acid. Ekatetrone and its triacetate were hydrolysed by alkali and dilute sulphuric acid, respectively, to an acidic compound $C_{19}H_{12}O_8$, which was readily esterified. The nature of the ultraviolet absorption spectrum and the conversion to anthracene by distillation with zinc dust suggested that it was an anthraquinone derivative. This was confirmed by detailed analysis of mass spectra, ^{1}H- and ^{13}C-nmr, and other spectroscopic data, which also enabled the definition of the nature and orientation of substituents.

The environment of each of the heteroatoms was readily defined by the spectroscopic data (see Experimental).

The nature of the functional group incorporating the nitrogen atom became evident mainly from the mass spectrum of ekatetrone. The loss of NH_3 from the molecular ion and an ion *m/e* 44, CH_2NO, suggested that a carboxamide group[4] was present. This deduction is supported by bands at 1675 and 3360 cm^{-1} in the infrared absorption spectrum; they are assignable to $\nu_{C=O}$ amide and ν_{N-H}, respectively. One of the signals in the 160~175 ppm region of the ^{13}C nmr spectrum of **I** (Table 1) can be attributed to the amide carbonyl. Furthermore, ekatetrone was converted to the ester **IV** through the corresponding acid **III**.

Information about the oxygen atoms was obtained indirectly using the ^{13}C nmr and infrared spectra. According to their chemical shifts, seven carbon atoms of ekatetrone (δ_C 189.0, 181.7, 170.6, 168.0, 165.2, 161.9 and 76.1) are attached to oxygen atoms. One (δ_C 76.1) is attributed to a sp^3 -hybridis-

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Table 1. ^{13}C nmr data of ekatetrone (I) and its triacetate (II).

I[a]						II[b]						
δ_C	mult.		^1J [Hz]	LS[e] [Hz]	f	δ_C	mult.		^1J [Hz]	LS[e] [Hz]	f	g
	c	d					c	d				
189.0	s	*				183.1	s	s				−5.9
181.7	s	*				181.7	s	t		3.9		0
						171.9	s	d		6.8		
170.6	s	t		4.9		170.6	s	*				0
						169.7	s	q		6.8		
168.0	s	d		1.8		169.3	s	q		6.8		
165.2	s	dd		2.4, 1.2		161.0	s	s				
161.9	s	t		5.5		155.0	s	q		2.9		
						150.3	s	dd		8.8, 2.9		
146.6	s	d		1.8		145.2	s	dd		7.8, 4.9		
146.6	s	d		1.8		138.4	s	s				
140.2	s	d		4.3								
137.0	d	dt	164.8	1.2	7.77 (W)	135.9	d	d	167.0		7.6 (W)	−1.1
133.0	s	*				134.6	s	d		6.8		
125.2	d	d	162.4	7.3	7.58 (V)	131.5	d	dd	167.0	6.8	7.98 (V)	6.3
						130.1	s					
121.2	s	*				127.0	s					
119.4	d	ddd	168.5	6.1, 1.8	7.31 (U)	125.8	d	dd	168.0	6.8	7.46 (U)	6.4
117.5	s	*				124.2	s	dd		6.8, 2.9		
114.8	d	dt	168.5	3.1	7.64 (X)	121.5	d	d	169.9	4.5[h]	7.74 (X)	6.7
76.1	d	dt	156.3	3.7	5.01 (E)	74.6	d	dt	145.0	3.5	5.26 (E)	1.5
					2.58						3.00	
40.6	t				2.83 (A,B)	40.0	t				3.17 (A,B)	0.6
					3.33						3.35	
31.8	t				4.17 (C,D)	32.6	t		132.8		3.83 (C,D)	−0.8
						25.6	q		128.9		2.21(NHAc)	
						21.7	q		129.3		2.34 (OAc)	
						21.7	q		129.3		2.42 (OAc)	

a) DMSO, 80°C b) DMSO, room temperature c) SFORD multiplicity d) proton coupled spectrum e) line splitting
f) ^{13}C-^1H cross-correlation, δ_H and its assigned symbol (see Fig. 1). g) acetylation shift, ppm h) half band width
* uncertain value, omitted

ed carbon atom of an oxymethine function; the other six are sp^2-hybridised carbon atoms. The first oxygen atom must be attributed to an oxymethine group. Two carbons (δ_C 189.0 and 181.7), carrying the second and third oxygen atoms, are assigned to a *para*-quinone system.[5] This accounts for the 1640 and 1660 cm^{-1} bands in the infrared spectrum. One of the seven carbon atoms and the fourth oxygen is part of the carboxamide group which has already been mentioned; this is responsible for the infrared band at 1675 cm^{-1}. The number of enolic groups attached to carbon atoms which resonate in the region 160~170 ppm may be deduced from the ^1H spectra of **II** and **IV** (Table 2). Two hydrogen atoms of enolic groups appear in the ^1H spectrum of the ester **IV** and only two singlets (δ_H 2.34 and 2.42) in ^1H nmr of the acetate (**II**) can be assigned to enol acetyls. There are, then two sp^2-carbon atoms carrying hydroxyl groups. The chemical shift of the last unassigned sp^2-carbon atom indicates that it may be due to a carboxyl, ester or lactone carbonyl.[6] Further evidence limits the explanation to a lactone group. First, it accounts for the 1705 cm^{-1} band in the infrared spectrum of **I**. Secondly, a carboxyl group is precluded by the hydrolysis and subsequent methylation experiment. The ^1H nmr spectrum of **IV** confirmed that it contained only one carbomethoxy group attributed to the original carboxamide function. None of the proton signals in the nmr spectra of the compounds **I**~**IV** can represent a separate terminal ester group. The conversion of the ion *m/e* 309 to the ion *m/e* 265 with loss of CO_2 is supported by the metastable peak at *m/e* 227. This is in accord with the known behaviour of lactones.[7]

Of the thirteen hydrogen atoms of ekatetrone, four are already attributed to two enolic hydroxyl groups and one $CONH_2$ group. There is also an nmr signal that could be attributed to a secondary

Table 2. ^1H nmr spectra of ekatetrone (**I**) and its derivatives (**II**~**IV**).

Proton	Compound			
	I[a]	II[b]	III[b]	IV[c]
A	2.58d (15.4)	3.00d (15)	2.86[g]	2.76dd (17.6, 2.0)
B	2.83d (15.4)	3.17d (15)	2.96[g]	3.16dd (17.6, 2.4)
C	3.33dd (18.4, 10.5)	3.35dd (15, 11)	4.33dd (14.7, 12.2)	3.36dd (18.6, 12.2)
D	4.17dd (18.4, 3.9)	3.83dd (15, 4)		4.42dd (18.6, 3.4)
E	5.01mt	5.26 mt	4.96 mt	5.01 mt (12.2, 3.4, 2.4, 2.0)
U	7.31dd (6.8, 2.9)	7.46dd (8, 2)	7.37dd (7.3, 2.4)	7.32dd (7.3, 2.4)
V	7.58dd (7.3, 2.9)	7.98dd (8, 2)	7.63dd (7.3, 2.4)	7.83dd (7.3, 2.4)
W	7.77t (7.3)	7.76t (8)	7.82t (7.3)	7.65t (7.3)
X	7.64s	7.74s	7.70s	7.88s
Others	12.43br s[d]	2.21[e] 2.34[e] 2.42[e]	12.55[d]	3.79s[f] 11.99s[d] 12.69s[d]

Splitting in Hz given in parentheses
s....singlet, d....doublet, t....triplet, br....broad
a: 80°C, 59.7974 MHz, DMSO b: 25°C, 59.7974 MHz, DMSO c: 25°C, 59.7971 MHz, $CDCl_3$
d: OH signal e: each 3H, Ac f: 3H, $COOCH_3$ g: inner lines of an AB-system

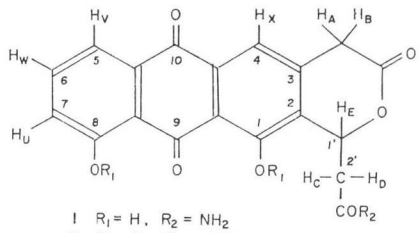
alcoholic group of **I**. There is a three-proton singlet at 2.21 ppm in the ^{1}H-nmr of the triacetate **II** which can arise from either O- or N-acetyl functions. However, the first possibility is excluded by the evidence derived from the mass spectrum of the triacetate (**II**). The diagnostic ion *m/e* 59 in **I** is shifted to *m/e* 101 ($C_4H_7NO_2$) for the derivative **II**.

The ion *m/e* 101 arises from the parent ion by two-stage elimination of ketene followed by the loss of the large aromatic fragment and itself loses the third unit for ketene to give the ion *m/e* 59. Evidently, one acetyl group in **II** is attached to nitrogen. This conclusion is supported by evidence of the loss of a $C_4H_7NO_2$ fragment from several ions in the course of the fragmentation of **II**. It was established by SFORD (Single Frequency Off-Resonance Decoupling) experiments that the remaining nine hydrogen atoms of ekatetrone were attached to carbon atoms in two aliphatic methylene groups, four sp^2-methines and one oxymethine group.

The ^{13}C nmr shift studies and the deductions which have been described indicate that there are two secondary sp^3, five tertiary (one sp^3-OCH and four sp^2-CH) and twelve quaternary (all sp^2-, six attached to oxygen, six to carbons only) carbon atoms. Consequently, the environment of each of the atoms in the molecular formula $C_{19}H_{13}NO_7$ has been interpreted.

Further evidence of structure was derived from the ^{1}H nmr spectral data (Table 2). The aromatic proton H_W (Fig. 1) with two *ortho*-couplings, is vicinal to the protons H_U and H_V which show mutual *meta*-coupling. The aromatic proton H_X is assinged to an isolated position. Both pairs of methylene protons (H_A, H_B and H_C, H_D) display large geminal couplings, indicating that they are close to at least one sp^2-hybridised carbon atom.[8] The protons H_C, H_D having one large (J_{CE}) and one medium size (J_{DE}) coupling, are vicinal to the oxymethine proton H_E. The protons H_A, H_B are attributed to an isolated methylene group. Their homoallylic couplings[9] to the proton H_E found in **I** and **IV** place both this methylene and the oxymethine *cis*- on the same double bond or at the vicinal positions on the same aromatic ring.

Fig. 1. Proton assignment in ekatetrone and its derivatives.



I R_1 = H, R_2 = NH_2
II R_1 = Ac, R_2 = NHAc
III R_1 = H, R_2 = OH
IV R_1 = H, R_2 = OMe

Cross-correlation of ^{13}C-and ^{1}H-nmr spectra[10] (Table 1) allows the comparison of ^{13}C nmr spectra of **I** and **II**. The observed acetylation shifts mean that carbon C_W is *meta* to an acetoxyl and the carbons C_U and C_V are *ortho* and *para* (or *vice versa*) to this group.[11] For the same reasons, the carbon C_X is either *ortho* or *para* to the second acetoxy group. The proton-coupled ^{13}C nmr spectrum of **II** establishes, unambiguously, that the quinone carbonyls have the relationship to the protonated carbons of a 1,8-dihydroxyanthraquinone. The downfield signal (δ_C 183.1) appears as a singlet and has therefore no protonated carbons in its *peri*-positions whereas the upfield one (δ_C 181.7) exhibits a triplet pattern indicating two such carbons. Since the first ring carries an OH group, the downfield carbonyl in **I** is hydrogen-bonded while the other is free. That agrees well with the observed chemical shifts (189.0 and 181.7 ppm) arising from analogous carbon atoms in such compounds as 1,8-dihydroxyanthraquinone,[12,13] deoxyaverufinone,[14] or nogalamycin.[15] As anticipated, the chemical shift of the free carbonyl group in ekatetrone is not changed by acetylation.

The location of the second enolic group is also established by means of the proton-coupled ^{13}C

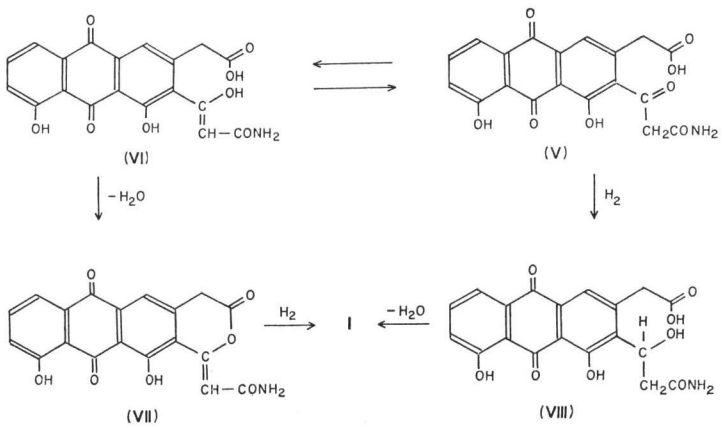
nmr spectra. The fine structure of the C_X-signal in **I** and **II** (doublet of triplets, and doublet with further unresolved splitting respectively) indicates that there is a methylene group associated with the *ortho*-position.[16,17] If there were a methine group the fine structure of this signal would be a doublet. The observed homoallylic couplings J_{AX} and J_{BX} in **IV** (Table 2) establish that the oxymethine group is vicinal to the CH_2 group and from knowledge of the environment of the other five oxygen atoms this one must be part of the lactone function.

The base peak in the mass spectrum of **I** has *m/e* 59 and the elemental composition C_2H_5NO. It contains the $CONH_2$ group and one methylene group. Another remarkable feature in the mass spectra of the compounds **I**~**IV** is the large intensity of the ion *m/e* 309. This ion arises from the elimination of a CH_2COR group, where R is NH_2, NHAc, OH or OMe. It follows that this CH_2COR group is a side chain. This makes easy the assignment of the methylene carbon atoms: the upfield signal (δ_C 31.8) is attributed to the side chain CH_2 bearing only one electronegative group ($CONH_2$). The downfield signal (δ_C 40.6) is due to a methylene group flanked by aromatic ring and another electronegative group, which, by elimination, must be the lactone function. This conclusion is confirmed by ^{13}C-^1H cross-correlation; the downfield methylene carbon does, indeed, carry the protons forming and isolated AB (H_A, H_B) system, whereas for the upfield methylene carbon atom the two protons (H_C, H_D) are coupled to the oxymethine function bearing the proton H_E. Therefore, the structure of ekatetrone is represented by the formula **I** (Fig. 1). The chiral carbon atom at position 1′ is responsible for the optical activity.

A Possible Biogenetic Relationship of Ekatetrone to Tetracyclines

The structure of ekatetrone bears an obvious relationship to protetrone (**V**), a metabolic product of a mutant of *Streptomyces aureofaciens*.[18] Evidently, protetrone could be transformed to ekatetrone (Fig. 2) either through lactonisation of the enolic form (**VI**) to give **VII** followed by reduction to **I** or through reduction to the carbinol (**VIII**) before lactonisation. There are various examples of such reductions by micro-organisms. The conversion of daunomycinone to the corresponding carbinol by the mutant strain *S. aureofaciens* B96, is typical.[19] The postulated connection between protetrone and the pathway of biosynthesis of tetracyclines links ekatetrone, also, to this group of metabolites of microorganisms.

Fig. 2. Protetrone-ekatetrone hypothetical relationship.



Experimental

General

Thin-layer chromatography (TLC) was performed on precoated silica gel sheets Silufol (Kavalier, Czechoslovakia) for qualitative analysis and Silufol 20 (Kavalier) for preparative studies. Plates with Kieselgel G (Merck) were used for both analytical and preparative separations. The following solvent systems were employed: S_1, chloroform - metanol 15 : 1; S_2, chloroform - methanol 9 : 1; S_3, chloroform - methanol 20 : 1; S_4, *n*-hexane.

Column chromatography was performed on silica gel CH (40~120 mesh, Lachema, Czechoslovakia).

Melting points were determined on a Boetius HMK apparatus and were not corrected. Specific rotation was measured at 25°C in dimethylformamide on Perkin-Elmer 141 MC apparatus.

UV and visible spectra (210~450 nm) were taken on Perkin-Elmer 412 or Cary 118 spectrophotometers in methanol or cyclohexane.

IR spectra were measured on Unicam SP 200 or UR 10 spectrophotometers in pellets (150 mg of potassium bromide and approx. 1.5 mg of sample).

The mass spectra were measured with a Varian MAT 311 mass spectrometer, using ionizing energy 70eV, ionizing current 1 mA, ion source temperature 200°C, direct inlet temperature 150~190°C. Elemental composition of all ions in fragmentation was confirmed with high resolution measurement. The high resolution mass spectrum was measured by peak matching technique with the inner standard perfluorokerosene (PFK). All the metastable transitions except *m/e* 309→265 of ekatetrone were confirmed with DADI technique. The metastable transition *m/e* 309→265 was observed in low resolution mass spectrum only (AEI MS9 instrument; 330°C). ^1H NMR and ^{13}C NMR spectra were measured on JEOL FX 60 (15 MHz-^{13}C, 59.79 MHz-^1H). Hexamethyldisiloxane (HMDS) served as an internal standard and the results were converted to the δ scale ($\delta_{HMDS}=0.06$); splitting is given in Hz.

Isolation of ekatetrone (I)

Ekatetrone used in our study was prepared according to the methods described in a previous paper[1] as a yellow crystalline solid, m.p. 270~272°C, $[\alpha]_D^{25}$ 304° (*c* 0.169, DMF). Anal. Calcd. for $C_{19}H_{13}NO_7$: C, 62.13; H, 3.57; N, 3.81. Found: C, 62.50; H, 3.77; N, 4.11. UV and visible spectrum λ_{max} (methanol) 227 (4.53), 252 (4.30), 276 (4.38), sh 315, 375 (3.77), 411 (3.64); nm (log ϵ). IR spectrum ν (KBr pellets) 3490, 3360, 1705, 1675, 1660, 1640, 1580; cm^{-1}. ^{13}C NMR spectrum (Table 1), ^1H NMR spectrum (Table 2). Mass spectrum *m/e* (elemental composition, relative intensity, list of daughter ions) 367 ($C_{19}H_{13}NO_7$, 16%, 350, 349, 309), 350 ($C_{19}H_{10}O_7$, 6%, 322), 349 ($C_{19}H_{11}O_6$, 6%), 322 ($C_{18}H_{10}O_6$, 9%), 309 ($C_{17}H_9O_6$, 85%, 291, 281), 291 ($C_{17}H_7O_5$, 54%, 263), 281 ($C_{16}H_9O_5$, 35%, 263 (253), 265 ($C_{16}H_9O_4$, 5%), 263 ($C_{16}H_7O_4$, 5%), 253 ($C_{15}H_9O_4$, 5%), 252 ($C_{15}H_8O_4$, 7%), 139 ($C_{11}H_7$, 21%), 59 (C_2H_5NO, 100%), 44 (CH_2NO, 22%).

Triacetate of ekatetrone (lactone of 1,8-diacetoxy-2(1′-hydroxy-2′-N-acetylcarbamoyl)ethyl-9,10-anthraquinone-3-acetic acid; II)

A suspension of ekatetrone (I) (50.2 mg) in acetic anhydride (8 ml) containing a catalytic amount of conc. sulfuric acid was heated at 100°C for 20 minutes. The acetate which was precipitated as a yellow solid by pouring the reaction mixture into distilled water (150 ml) was filtered, washed with distilled water and dried (53.6 mg). The crude product was dissolved in chloroform (10 ml) and purified by column chromatography (1.1 × 28 cm). The column was developed with chloroform and fractions of 15 ml were collected. The residue obtained by evaporating the combined fractions 9~11 was dissolved in chloroform (10 ml), filtered and diluted with diethyl ether (4 ml). The mixture was allowed to stand at laboratory temperature (10 hours). The triacetate of ekatetrone crystallized as yellow needles. The product was washed with a mixture of chloroform - diethyl ether (1 : 2). The yield of the triacetate was 44.9 mg (80.3%), m.p. 198~203°C. TLC (Silufol, solvent system S_1, Rf 0.65).

UV and visible spectrum λ_{max} (methanol) 220 (4.14), 256 (4.15), 345 (3.64); nm (log ϵ). IR spectrum ν(KBr pellets) 3370, 1765, 1725, 1705, 1680, 1590; cm^{-1}. ^{13}C NMR spectrum (Table 1),

^{1}H NMR spectrum (Table 2). Mass spectrum *m/e* (elemental composition, relative intensity, list of daughter ions) 493 ($C_{25}H_{19}NO_{10}$, 0.04%, 475, 451, 392), 475 ($C_{25}H_{17}NO_9$, 0.08%, 433, 374), 451 ($C_{23}H_{17}NO_9$, 3%, 433, 409, 350), 433 ($C_{23}H_{15}NO_8$, 3%, 332), 409 ($C_{21}H_{15}NO_8$, 8%, 367, 101), 392 ($C_{21}H_{12}O_8$, 4%, 374, 350), 374 ($C_{21}H_{10}O_7$, 2%, 332), 367 ($C_{19}H_{13}NO_7$, 2%, 350, 309), 351 ($C_{19}H_{11}O_7$, 10%), 350 ($C_{19}H_{10}O_7$, 11%, 322), 332 ($C_{19}H_8O_6$, 1%), 322 ($C_{18}H_{10}O_6$, 11%), 309 ($C_{17}H_9O_6$, 79%, 291, 281), 291 ($C_{17}H_7O_5$, 38%, 263), 281 ($C_{16}H_9O_5$, 15%, 263, 252), 265 ($C_{16}H_9O_4$, 4%), 263 ($C_{16}H_7O_4$, 3%), 252 ($C_{15}H_8O_4$, 3%), 139 ($C_{11}H_7$, 2%), 101 ($C_4H_7NO_2$, 38%, 73, 59), 73 (C_3H_7NO, 7%), 60 ($C_2H_4O_2$, 19%), 59 (C_2H_5NO, 35%), 43 (C_2H_3O, 100%), 42 (C_2H_2O, 46%).

Ekatetrone acid (lactone of 1,8-dihydroxy-2(1′-hydroxy-2′-carboxy)ethyl-9,10-anthraquinone-3-acid; **III**)

(a) Ekatetrone (**I**) (50 mg) was dissolved in 10% aqueous sodium hydroxide (30 ml) and methanol (30 ml). The reaction mixture was heated under nitrogen 4 hours at 80°C. After cooling, the pH was adjusted to 3 using 1 N hydrochloric acid. The product of the reaction was extracted with chloroform (4×80 ml) and the extracts were pooled, extracted with water (2×100 ml) and dried with anhydrous sodium sulfate. The chloroform extract was concentrated to a volume of 15 ml and purified by column chromatography (1.2×30 cm). The column was developed with chloroform and 10 ml fractions were collected. Fractions 8~15 were pooled and reduced to 5 ml. The ekatetrone acid was precipitated by addition of petroleum ether (3 ml). The yellow precipitate was separated by suction and washed with petroleum ether. The yield of ekatetrone acid after recrystallization from chloroform-*n*-hexane was 8.5 mg (16.9%). M.p. 197~201°C, TLC chromatography (Kieselgel G, solvent system S_2, Rf 0.6).

UV and visible spectrum λ_{max} (methanol) 229 (4.54), sh 255, 278 (4.45), sh 315, 410 (3.83); nm (log ϵ). IR spectrum ν (KBr pellets) 1722 ($\Delta\nu_{1/2}=25$ cm^{-1}), 1678, 1632, 1574; cm^{-1}. ^{13}C NMR spectrum (Table 1), ^{1}H NMR spectrum (Table 2). Mass spectrum *m/e* (elemental composition, relative intensity, list of daughter ions) 368 ($C_{19}H_{12}O_8$, 25%, 322, 309), 322 ($C_{18}H_{10}O_6$, 5%), 309 ($C_{17}H_9O_6$, 100%, 291, 281), 291 ($C_{17}H_7O_5$, 24%, 263), 281 ($C_{16}H_9O_5$, 48%, 263, 253), 265 ($C_{16}H_9O_4$, 3%), 263 ($C_{16}H_7O_4$, 2%), 253 ($C_{15}H_9O_4$, 4%), 252 ($C_{15}H_8O_4$, 5%), 139 ($C_{11}H_7$, 9%).

(b) A suspension of ekatetrone triacetate (**II**) (26 mg) in 30% sulfuric acid (10 ml) was boiled for half an hour. After cooling to 50°C, 10% aqueous sodium nitrite was introduced to the bottom of the reaction flask. After the reaction had proceeded for 10 minutes, distilled water (20 ml) was added and the mixture was extracted with chloroform (40 ml). The extract was washed with water (2×40 ml), dried and concentrated to 2 ml. This concentrate was purified by preparative TLC (Silufol 20, solvent system S_3). Ekatetrone acid (**III**) was eluted with chloroform from the main band on the plate (14.9 mg). Recrystallization from chloroform-*n*-hexane yielded 11.5 mg of ekatetrone acid (**III**) with the same characteristics as in (a).

Methylester of ekatetrone acid (lactone of 1,8-dihydroxy-2(1′-hydroxy-2′-methoxycarbonyl) ethyl-9,10-anthraquinone-3-acetic acid; **IV**)

The suspension of ekatetrone acid (**III**) (11.5 mg) in boron trichloride-methanol (10 ml) was heated at 80°C for 45 minutes. The reaction mixture was hydrolyzed with water (10 ml, 5 minutes). The methyl ester was extracted with chloroform (2×20 ml). This extract was washed with water (2×30 ml), and concentrated to 3 ml. This concentrate was purified by preparative TLC (Silufol 20) in the solvent system S_3. The main band was eluted with chloroform (2×10 ml) and the yield of methyl ester of ekatetrone acid (**IV**) after recrystallization from chloroform - *n*-hexane was 4.5 mg, yellow needles m.p. 190~194°C. TLC (Silufol) reveals a single spot Rf 0.65 in the solvent system S_3.

UV and visible spectrum λ_{max} (methanol) 227 (4.31), 253 (4.06), 276 (4.12), 377 (3.44), 410 (3.46); nm (log ϵ). IR spectrum ν (KBr pellets) 1742 ($\Delta\nu_{1/2}=12$ cm^{-1}), 1690, 1675, 1632, 1573; cm^{-1}. ^{13}C NMR spectrum (Table 1), ^{1}H NMR spectrum (Table 2). Mass spectrum *m/e* (elemental composition, relative intensity, list of daughter ions) 382 ($C_{20}H_{14}O_8$, 26%, 364, 351, 322), 364 ($C_{20}H_{12}O_7$, 1%, 291), 351 ($C_{19}H_{11}O_7$, 2%, 309), 322 ($C_{18}H_{10}O_6$, 7%), 309 ($C_{17}H_9O_6$, 100%, 291, 281), 291 ($C_{17}H_7O_5$, 57%, 263), 281 ($C_{16}H_9O_5$, 36%, 263, 253), 265 ($C_{16}H_9O_4$, 2%), 263 ($C_{16}H_7O_4$, 2%), 253 ($C_{15}H_9O_4$, 3%), 252 ($C_{15}H_8O_4$, 6%), 139 ($C_{11}H_7$, 8%), 74 ($C_3H_6O_2$, 4%).

Reduction of ekatetrone (I) with zinc dust to anthracene

A mixture of ekatetrone (I) (100 mg) with zinc dust (1 g), zinc chloride (1 g) and sodium chloride (100 mg) in a tube (diam. 6 mm, length 20 cm) was heated in an oven to 300°C. After 20 minutes a brown-yellow sublimate was formed on the cool part of the tube. The sublimate was transferred into benzene (2 ml) and purified by column chromatography (1 × 20 cm). The column was developed with chloroform. The fractions containing anthracene were monitored by TLC on Kieselgel G in the solvent system S_4. The band of anthracene (Rf 0.4, blue fluorescence in UV light, 254 nm) was eluted with chloroform and the solution evaporated. The residue was sublimed under reduced pressure (water pump) at the temperature of 110~115°C. The yield of anthracene was 2.7 mg (5.5%), m.p. 215°C, mixed m.p. with authentic anthracene showed no depression, UV and visible spectrum λ_{max} (cyclohexane) 221 (4.29), 246 (4.99), 253 (5.33), 291 (2.94), 309 (3.13), 323 (3.48), sh 335, 339 (3.76), sh 352, 357 (3.94), sh 371, 376 (3.92); nm (log ϵ). The mass spectrum M^+ 178, $C_{14}H_{10}$.

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