METABOLIC PRODUCTS OF MICROORGANISMS. 208[1] HALOQUINONE, A NEW ANTIBIOTIC ACTIVE AGAINST HALOBACTERIA

II. CHEMICAL STRUCTURE AND DERIVATIVES

BERND KRONE, ANKE HINRICHS and AXEL ZEECK*

Organisch-Chemisches Institut, Universität Göttingen
Tammannstr. 2, D-34 Göttingen, W. Germany

(Received for publication July 6, 1981)

Evidence is put forward, which describes the structure of haloquinone as 3-acetyl-1,8-dihydroxy-2-methyl-9,10-phenanthrenequinone (**1a**).

In the preceding paper[1] the isolation and characterization of the new antibiotic haloquinone, produced by *Streptomyces venezuelae* ssp. *xanthophaeus* were described. In the following we report the structural data and some chemical reactions of the antibiotic.

Mass spectral and elemental analysis of haloquinone (**1a**) are in agreement with the formula $C_{17}H_{12}O_6$. The presence of two *C*-methyl groups is proved by KUHN-ROTH oxidation. Haloquinone is an indicator substance. The dark red antibiotic gives a blue solution in NaOH, the color immediately turns yellow by reducing it with zinc dust, the reduced form is reoxidized by air. This behaviour is typical for a hydroxyquinone. This observation is supported by the fact that haloquinone forms a blue-green color in 0.001 N ethanolic magnesium acetate or, after heating, in acetic anhydride/pyroboracetate. The pigment is insoluble in sodium hydrogencarbonate solution and does not react quickly with diazomethane at room temperature. Evidently there are no carboxy or unchelated phenolic hydroxyl groups. A characteristic reaction of haloquinone is the quick (1 minute) decolorization of its alkaline solution with hydrogen peroxide caused by the ortho-quinone structure (see below).

The spectral data of haloquinone (**1a**) are very similar to those of piloquinone (**2a**), isolated from *Streptomyces pilosus*[2,3,4]. Comparisons of the UV and the PMR data are given in Tables 1 and 2. The PMR-signals of the side chain of **2a** are missing in the spectrum of haloquinone (**1a**).

The chemical reactions of haloquinone (**1a**) and piloquinone (**2a**) are quite similar[2,3], *i.e.* with *o*-phenylenediamine the yellow quinoxaline-derivative **3** can be obtained, which proves the ortho-quinone structure. The reaction of haloquinone with methyliodide/silver(I)-oxide leads to the yellow dimethyl ether **1b**, acetylation with acetic anhydride/sodium acetate yields the unstable yellow diacetate **1c**. The colorless leucotetraacetate **4** is prepared by reductive acetylation. The deoxydihydro derivative **6**, which contains an ethyl group instead of the acetyl side chain is obtained by CLEMMENSEN reduction. These derivatives of haloquinone are characterized in the experimental part. Their Rf values are given in Table 3.

Table 1. UV spectra (ethanol) of haloquinone (**1a**) and piloquinone (**2a**).[2,3]

	λ_{max} (ε) nm
1a	512 (6000), 395 (4300), 284 (20800), 277 sh, 232 (34300)
2a	515 (5560), 396 (4075), 286 (17000), 277 sh, 233 (32000)

* To whom communications should be addressed.

Table 2. PMR signals ($CDCl_3$, δ in ppm) of haloquinone (**1a**), its bromo derivatives **5a** and **5b** and piloquinone (**2a**).[4]

H-atom	**1a**, 250 MHz	**5a**, 100 MHz	**5b**, 100 MHz	**2a**, 60 MHz
4-H	7.45 s	7.46 s	8.57 s	7.30 s
5-H	7.47 dd[a]	7.40 d[b]	—	7.37 dd
6-H	7.59 dd[b]	7.88 d[b]	8.22 s	7.58 dd
7-H	7.00 dd[a]	—	—	6.93 dd
2-CH_3	2.33 s	2.34 s	2.38 s	2.23 s
3-$COCH_3$	2.63 s	2.65 s	2.64 s	—
1-OH	12.83 s	12.95 s	13.27 s	12.75 s
8-OH	12.36 s	12.81 s	12.67 s	12.28 s

[a] J=8.0 and 1.5 Hz, [b] J=8.0 Hz.

The mass spectrum (Fig. 1) of haloquinone (**1a**) shows a strong molecular ion (*m/z* 296, 82% relative intensity). The base peak occurs at *m/z* 253 by loss of $COCH_3$. The loss of CH_3 can also be observed (*m/z* 281, 5%). Typical for the quinone structure is the extrusion of CO and 2 CO (peaks at *m/z* 268 and 240), which is followed by the loss of $COCH_3$ (*m/z* 225 and 197).

The PMR spectrum (Table 2) of haloquinone (**1a**) shows signals for two chelated phenolic hydroxyl groups as sharp singlets at δ 12.83 and 12.36, which vanish after addition of CD_3OD. These groups take the *peri*-positions to the two quinone carbonyls. Four aromatic protons give signals in the region δ 7.6~7.0, a singlet is to be seen at δ 7.45 together with an ABC-coupling system for three H-atoms, adjacent to each other at the other aromatic ring. The singlet at δ 2.63 is attributed to the acetyl group, an aromatic methyl group exhibits a broadened singlet at δ 2.33. The PMR data clarify that the methyl and acetyl group cannot be located in different rings.

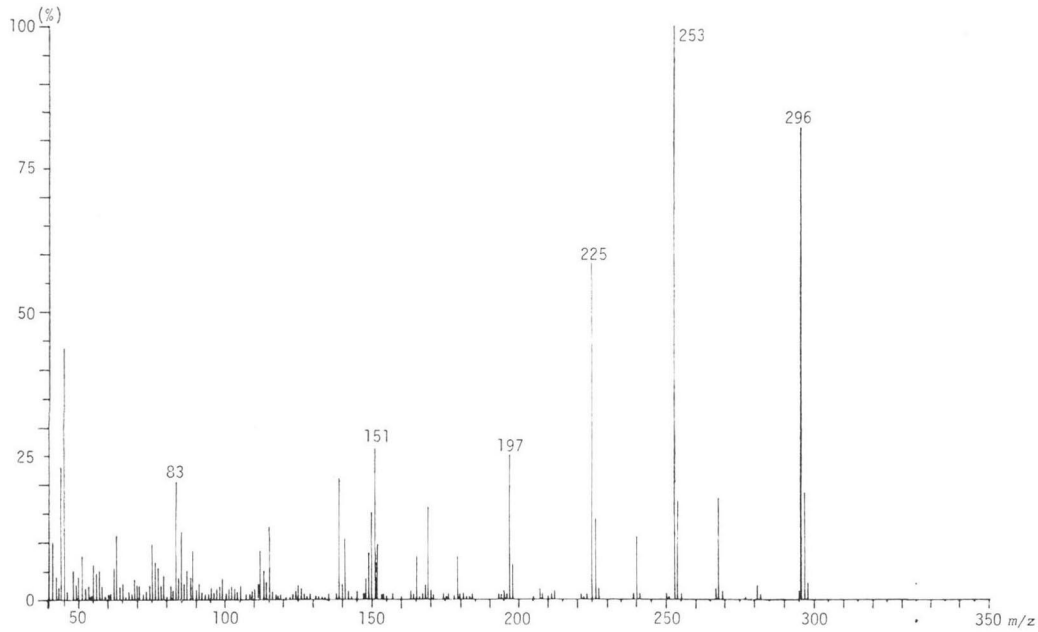
Table 3. Rf values of haloquinone (**1a**) and some derivatives on silica gel (TLC sheets aluminium, Woelm F 254/366, treated with oxalic acid) in chloroform - acetone (97: 3).

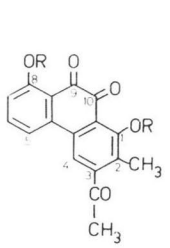
Compound	Color	Rf
Haloquinone (**1a**)	red	0.48
Haloquinone-1,8-dimethyl ether (**1b**)	yellow	0.15
7-Bromohaloquinone (**5a**)	red	0.53
5,7-Dibromohaloquinone (**5b**)	red	0.59
Haloquinone quinoxaline (**3**)	yellow	0.56
Haloquinone leucotetra-acetate (**4**)	colorless	0.19
Deoxydihydrohaloquinone (**6**)	red	0.58

Further derivatization, *i.e.* bromination of haloquinone was accomplished with 1,4,4,6-tetrabromocyclohexa-2,5-dien-1-one[5] yielding the 7-bromohaloquinone (**5a**) and the 5,7-dibromohaloquinone (**5b**) in a ratio dependent on the excess of the reagent and on the reaction time. The two compounds were separated by column chromatography on silica gel. The position of the bromine atoms results from the δ values and the coupling constants of the remaining aromatic H-atoms in the PMR spectrum (Table 2). The ABC-coupling system of the three adjacent aromatic H-atoms in haloquinone (**1a**) is simplified to an AB system (J=8.0 Hz) in **5a**. The H-atom of the other aromatic ring remains unaffected. In the dibromo derivative **5b**, however, a remarkable paramagnetic shift of this proton is observed due to the influence of a bromine atom at C-5. A similar effect ($\Delta\delta$ 1.39) is reported for 4-bromophenanthrene compared with phenanthrene[6]. These results prove the position of the H-atom attached at ring A as well as the position of the bromine atoms in **5a** and **5b**.

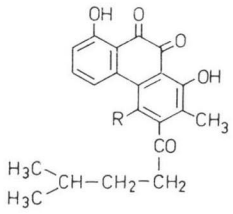
Haloquinone itself is not soluble enough to obtain an efficient ^{13}C NMR spectrum; the haloquinone dimethyl ether (**1b**) was sufficiently soluble. Beside two quinone carbonyl groups (δ 182.4, 181.6) it gave

Fig. 1. Mass spectrum (70 eV) of haloquinone (**1a**).

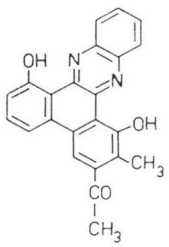




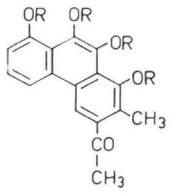
1a R = H
1b R = CH_3
1c R = CH_3CO



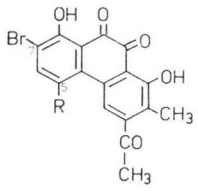
2a R = H
2b R = OH



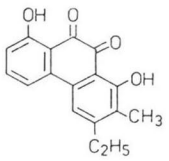
3



4 R = CH_3CO



5a R = H
5b R = Br



6

signals for twelve aromatic C-atoms, four of them doublets in the off-resonance spectrum (δ 112.7, 116.6, 119.0, 119.1, for C-4, C-5, C-6 and C-7); the others singlets (δ 124.5, 132.1, 135.6, 136.5, 137.6, 146.6, 161.0 and 161.7). The methoxy group resonances are at δ 61.8 (1-OCH_3) and 56.2 (8-OCH_3). The signals of the acetyl group (δ 30.3/201.6) are shifted downfield, compared with acetophenone (δ 24.6/

196.3), indicating that the acetyl group is located beside an *ortho*-substituent[7]. Most remarkable is the chemical shift of the aromatic methyl group at δ 12.7. The high field position cannot be explained by the neighbourhood of only one *ortho*-substituent (*o*-cresol: δ 17.9; 2-methylacetophenone: δ 20.6). However, a high field shift for signals of aromatic methyl groups is observed if two *ortho*-substituents are present, for example in 2,3-dimethylanisole δ 10.9[8]. The CMR data thus make evident that the aromatic methyl group of haloquinone is located between the hydroxyl and the acetyl group in ring A. The position of the methyl group for piloquinone (**2a**) has been defined by zinc dust distillation, which afforded phenanthrene and 2-methylphenanthrene[3].

The biosynthesis of haloquinone (**1a**) should proceed in analogy to piloquinone (**2a**)[9] from acetate *via* the polyketide pathway but with acetate as starter unit. Beside piloquinone (**2a**) *Streptomyces pilosus* produces 4-hydroxypiloquinone (**2b**)[10]. Some minor and biologically inactive pigments of the haloquinone producing strain remain to be examined.

Ortho-quinones, produced by microorganisms are very rare; worth mentioning are mycochrysone[11], olivovarin[12] and β-rubromycin[13~15]. None of these is a phenanthrenequinone.

Experimental

General

UV spectra were recorded using a Zeiss DMR 21 spectrometer, IR spectra in pressed KBr disks using a Perkin-Elmer model 298 spectrometer. PMR spectra were determined at 80 MHz with a Varian FT-80, at 100 MHz with a Varian HA-100 resp. Varian XL-100 or at 200 MHz with a Varian XL-200. Chemical shifts (δ in ppm) are reported relative to internal TMS. EI-mass spectra were taken with a Varian MAT-731 instrument (70 eV), high resolutions with perfluorokerosine as a standard. All molecular formulae were in agreement with the high resolutions. The FT-CMR spectrum was measured at 25.2 MHz with a Varian XL-100 spectrometer using TMS as internal standard (δ 0 ppm). Melting points were determined with a heated microscope (Reichert, Austria). For chromatography on columns or plates (20×40 cm) we used oxalic acid treated silica gel (Machery & Nagel, <0.07 mm). All eluates containing oxalic acid were extracted three times with water and dried with sodium sulfate (anhydrous). All evaporations were carried out under diminished pressure. Thin-layer chromatography (TLC) was performed on silica gel (aluminum sheets, Merck 60 F 254 or Woelm $F_{254/366}$), treated with 0.25 M oxalic acid, dried at room temperature and activated for 1 hour at 100°C.

Haloquinone-quinoxaline (3)

An ethereal solution of **1a** (10 mg), 1,2-diaminobenzene (20 mg), sodium sulfate (anhydrous, 20 mg) and 10 drops of acetic acid were left to stand for 3 hours at room temperature. The yellow mixture was washed once with 0.1 N NaOH and once with 0.1 M H_2SO_4. The dried solution was evaporated yielding 6.5 mg (52%) of pure **3**. IR (KBr): 1706, 1670 cm^{-1}. UV ($CHCl_3$): λ_{max} 435, 335 sh, 315, 267, 252 nm. PMR (100 MHz, $CDCl_3$): δ 2.58 (s, 2-CH_3), 2.76 (s, 3-$COCH_3$), 7.2~8.5 (complex, 8 H), 14.71/15.30 (s, 1-OH/8-OH). MS (70 eV): *m/z* (abund.)=368 (100%, $M^{+\cdot}$, $C_{23}H_{16}N_2O_3$), 353 (11%, M−CH_3), 340 (17%, M−CO), 325 (54%, M−CH_3−CO).

3-Acetyl-1,8-dimethoxy-2-methyl-9,10-phenanthrenequinone (1b)

1a (74 mg, 0.25 mmole) was dissolved in a large excess of methyl iodide (40 ml), then 0.2 g (0.86 mmole) silver (I)-oxide was carefully added. The reaction was carried out at room temperature under stirring. TLC showed a disappearance of **1a** after 2 hours. The mixture was filtered and evaporated to afford a yellow residue which was purified by dropping a concentrated chloroform solution into *n*-pentane. The precipitate was collected and yielded 78 mg (96%) **1b**, mp 192°C. IR (KBr): 1689, 1672, 1588, 1578 sh cm^{-1}. UV (MeOH): λ_{max} (ε) 430 (2800), 350 (3800), 253 (22000), 223 nm (31500). PMR (200 MHz/$CDCl_3$): δ 2.38 (s, 2-CH_3), 2.64 (s, 3-$COCH_3$), 3.88 (s, 8-OCH_3), 3.98 (s, 1-OCH_3), 7.02 (dd, 7-H), 7.51 (dd, 5-H), 7.63 (dd like t, 6-H), 7.75 (s, 4-H), $J_{5,6}=J_{6,7}=8.0$ Hz and $J_{5,7}=ca.$ 1.0 Hz.

MS (70 eV): m/z (abund.)=326 (48%, M+2), 324 (100%, $M^{+\cdot}$, $C_{19}H_{16}O_5$), 311 (31%, M+2−CH_3), 309 (7%, M−CH_3), 296 (70%, M−CO), 281 (95%, M−CO−CH_3).

Anal. Calcd. for $C_{19}H_{16}O_9$: C 70.36; H 4.97; 2 CH_3O 19.1%
Found: C 70.17; H 4.98; CH_3O 19.3%

1,8,9,10-Tetraacetoxy-3-acetyl-2-methylphenanthrene (**4**)

A solution prepared from 30 mg of **1a** in 10 ml acetic anhydride - pyridine (3: 2) was stirred at 40°C. When after 10 minutes the yellow diacetate **1c** had been formed, 120 mg of 5% palladium on carbon were added and hydrogenation with normal pressure hydrogen was performed. After 1 hour the catalyst was filtered off and the filtrate was poured into 200 ml ice water. After 18 hours the aqueous solution was extracted with chloroform, the organic layer was washed with 0.5 N HCl and water. Evaporation gave a residue which was purified by chromatography on silica gel plates (20×40 cm, 0.5 mm) with chloroform - acetone (97: 3). Two fractions containing colorless substances were obtained (detected in TLC by UV light). The fraction with the slower running substance was taken to dryness and gave 4 mg (9%) **4**, mp 225°C. IR (KBr): 1772, 1687, 1615, 1607 cm^{-1}. UV (MeOH): λ_{max} (ε) 313 (12000), 255 (45500), 219 nm (82000). PMR (100 MHz, $CDCl_3$): δ 2.39, 2.41, 2.42, 2.43, (s, 15 H, 4OCOCH_3 and 2-CH_3), 2.87 (s, COCH_3), 7.28 (dd, J=8.0 and 1.0 Hz, 7-H), 7.68 (dd like t, 6-H), 8.57 (dd, 5-H), 8.86 (s, 4-H). MS (70 eV): m/z (abund.)=466 (3%, $M^{+\cdot}$, $C_{25}H_{22}O_9$), 424 (1%, M−CH_2CO), 382 (4%, M−2×CH_2CO), 364 (23%), 340 (15%, M−3×CH_2CO), 322 (19%), 298 (100%, M−4×CH_2CO).

Bromination of Haloquinone (**1a**)

A stirred solution of 20 mg (0.1 mmole) of **1a** in 20 ml chloroform was treated with 60 mg (0.15 mmole) of 2,4,4,6-tetrabromocyclohexa-2,5-dienone at room temperature. TLC showed the appearance of two new compounds running quicker than **1a**. After 2.5 hours insoluble material was removed by filtration and the filtrate concentrated to dryness. The residue was chromatographed on a column of oxalic acid-treated silica gel. Eluting with chloroform - acetone (97: 3) two fractions (B before A) were collected, washed with water and taken to dryness.

Fraction A gave 17 mg (38%) red 3-acetyl-7-bromo-1,8-dihydroxy-2-methyl-9,10-phenanthrenequinone (**5a**), mp 235°C. IR (KBr): 1700, 1624 cm^{-1}. UV (MeOH, red): λ_{max} (ε) 510 (11300), 397 (7000), 286 (36500), 277 (35800), 232 nm (57000). UV (MeOH - NaOH, blue): λ_{max} (ε) 580 (18500), 450 (6800), 300 nm (12300). PMR see Table 2. MS (70 eV): m/z (abund.)=376/374 (100%, $M^{+\cdot}$, $C_{17}H_{11}O_5Br$), 348/346 (34%, M−CO), 333/331 (98%, M−CH_3CO), 320/318 (12%, M−2×CO), 305/303 (60%, M−CH_3CO−CO), 277/275 (17%, M−CH_3CO−2×CO).

Fraction B gave 8 mg (21%) of red 3-acetyl-5,7-dibromo-1,8-dihydroxy-2-methyl-9,10-phenanthrenequinone (**5b**), mp. 198°C (dec.). IR (KBr): 1700, 1630 cm^{-1}. UV (MeOH-HCl): λ_{max} (ε) 505 (6300), 405 (5000), 289 (15500), 239 nm (35300). UV (MeOH-NaOH, blue violet): λ_{max} (ε) 555 (8400), 440 (5500), 243 nm (35700). PMR data see Table 2. MS (70 eV): m/z (abund.)=456/454/452 (75%, $M^{+\cdot}$, $C_{17}H_{10}O_5Br_2$), 426 (41%, M−CO), 411 (100%, M−CH_3CO), 383 (44%, M−CH_3CO−CO).

3-Ethyl-1,8-dihydroxy-2-methyl-9,10-phenanthrenequinone (**6**)

To a suspension of **1a** (30 mg) in 10 ml dry ethanol 240 mg of zinc dust was added. Under stirring and heating (70°C) 0.48 ml conc. HCl was slowly dropped into the mixture. The insoluble material was filtered off and the filtrate was left in air until the color had turned from yellow to red again. The residue obtained by evaporation was dissolved in chloroform which was washed with water, dried and evaporated to dryness. The dark red residue was separated on silica gel plates (20×40 cm, 0.5 mm) by developing with chloroform - acetone (97: 3). The quickest of five red zones gave 8 mg (28%) **6** which was precipitated from a chloroform solution pouring into *n*-pentane. Mp 182~184°C (decoloration). IR (KBr): 1618, 1575 cm^{-1}. UV (MeOH): λ_{max} (ε) 510 (4100), 395 (3800), 300 sh, 283 (18400), 260 (15400), 225 nm (37000). UV (MeOH-NaOH): λ_{max} (ε) 575 (7200), 300 (10300), 289 nm (11800). PMR (80 MHz, $CDCl_3$): δ 1.25/2.67 (t/q, J=7.5 Hz, 3-CH_2CH_3), 2.19 (s, 2-CH_3), 6.85 (dd, J=8.0 and 1.5 Hz, 7-H), 7.20~7.60 (m, 2 H, 5-H and 6-H), 7.40 (s, 4-H), 12.22 (s, 8-OH), 12.71 (s, 1-OH). MS (70 eV): m/z (abund.) 282 (100%, $M^{+\cdot}$, $C_{17}H_{14}O_4$), 254 (80%, M−CO), 253 (22%, M−C_2H_5), 239 (30%, M−CO−CH_3), 225 (25%, M−CO−C_2H_5), 211 (19%, M−CH_3−2×CO).

Acknowledgement

We are grateful to Prof. H. ZÄHNER for providing us with haloquinone and to Dr. J. KURZ (Bayer AG, Wuppertal) for the 250 MHz PMR spectrum of haloquinone. We thank the Deutsche Forschungsgemeinschaft for generous financial support.

References

1) EWERSMEYER-WENK, B.; H. ZÄHNER, B. KRONE & A. ZEECK: Metabolic products of microorganisms. 207. Haloquinone, a new antibiotic active against halobacteria. I. Isolation, characterization and biological properties. J. Antibiotics 34: 1531~1537, 1981
2) JOHNSON, B. C.; P. COHEN, J. POLONSKY & E. LEDERER: Piloquinone: a new phenanthrene-*o*-quinone isolated from the mycelium of *Streptomyces pilosus*. Nature 199: 285~286, 1963
3) POLONSKY, J.; P. COHEN & E. LEDERER: Sur la structure chimique de la piloquinone, nouvelle *o*-phénanthrènequinone isolée de *Streptomyces pilosus*. Bull. Soc. Chim. France 1963: 1909~1917, 1963
4) GAUDEMER, A.; J. POLONSKY & L. ALAIS: Spectres de resonance magnétique nucléaire de la piloquinone et de ses dérivés. Bull. Soc. Chim. France 1963: 1918~1922, 1963
5) CALÓ, V.; F. CIMINALE, L. LYPEZ & P. E. TODESCO: A selective bromination of aromatic amines. J. Chem. Soc. (C) 1971: 3652~3653, 1971
6) BERINGER, F. M.; L. L. CHANG, A. N. FENSTER & R. R. ROSSI: Synthesis of 4-halophenanthrenes and 4,5-phenanthryleneiodonium salts. Tetrahedron 25: 4339~4345, 1969
7) DHAMI, K. S. & J. B. STOTHERS: ^{13}C-NMR studies. III. Carbon-13 NMR spectra of substituted acetophenones. Can. J. Chem. 43: 479~497, 1965
8) NETZEL, D. A.: Proximity and the heteroatom effects on the carbon 13 chemical shifts of methyl-substituted phenols, anilines and thiophenols. Org. Magn. Reson. 11: 58~68, 1978
9) ZYLBER, J.; E. ZISSMANN, J. POLONSKY & E. LEDERER: Sur la biosynthèse de la piloquinone, *o*-phénanthrènequinone produite par *Streptomyces pilosus*. European J. Biochem. 10: 278~283, 1969
10) LOUNASMAA, M. & J. ZYLBER: Sur l'isolement et la structure de l'hydroxy-4-piloquinone, deuxième *o*-phénanthrènequinone de *Streptomyces pilosus*. Bull. Soc. Chim. France 1969: 3100~3103, 1969
11) READ, G.; A. RASHID & L. C. VINING: Quinone epoxides. IV. Structure and stereochemistry of mycochrysone. J. Chem. Soc. (C) 1969: 2059~2068, 1969
12) VESELOVA, S. I.; M. N. GROMOVA, G. B. LOKSHIN, R. KH. ZABIROVA & A. D. KUZOVKOV: Production of *Actinomyces olivovariabilis* sp. nov. mutants which are variamycin producers and a study of their biosynthetic products. I. Two independent mutants forming olivovariin. Genetika 12: 122~128, 1976
13) BROCKMANN, H.; W. LENK, G. SCHWANTJE & A. ZEECK: Rubromycine. II. Chem. Ber. 102: 126~151, 1969
14) BROCKMANN, H. & A. ZEECK: Rubromycine. III. Die Konstitution von α-Rubromycin, β-Rubromycin, γ-Rubromycin and γ-iso-Rubromycin. Chem. Ber. 103: 1709~1726, 1970
15) DE BARROS COELHO, J.S.; O. GONCALVES DE LIMA, F. DELLE MONACHE, F. FERRARI & G.B. MARINI-BETTOLO: Identification of tuoromycin with β-rubromycin. Farmaco Ed. Sci. 25: 721~726, 1970