EPIMER OF 7-DEOXYAKLAVINONE

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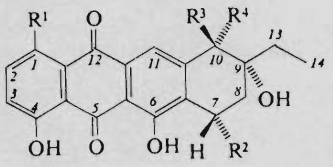
The structure (9*R*, 10*S*)-7-deoxyaklavinone (*VII*) was assigned to a minor metabolite isolated from *Streptomyces coeruleorubidus* on the basis of ^{1}H-, ^{13}C-NMR and CD spectra. Evidence that compounds *II* and *VII*, isomeric with 7-deoxyaklavinone, are natural compounds is presented.

7-Deoxyderivatives of anthracyclinones[1] belong to the common congeners of glycosidic antibiotics of anthracycline series[2,3], produced by some strains of *Streptomyces*. They are even the prevailing metabolites in cultures grown under anaerobic conditions or in certain mutant strains. Their formation by catalytic hydrogenolysis of both glycosides and aglycones is widely employed during the structure elucidation. These compounds are often formed by biotransformation of anthracyclinones and anthracyclines[5,4]. In previous work[6], we had isolated from a mutant strain of *Streptomyces coeruleorubidus* 7-deoxyaklavinone (*I*) besides its tricyclic isomer *II*. In the mother liquors from the crystallization of *I* we now found another its isomer, *VII*. Since *II* could be either a precursor of *I* or a product of its retroaldolization, we dealt with the elucidation of mutual relationships of all three compounds.

The investigated minor compound has an elemental composition $C_{22}H_{20}O_7$ (high resolution mass spectroscopy). Its mass, infrared, and UV/VIS spectra differ only slightly from that of 7-deoxyaklavinone *I*. The similarity with *I* is further emphasized by the ^{1}H- and ^{13}C-NMR spectra (Table I, Fig. 1). Both compounds have the same distribution of protons and carbons (type and number of nuclei of the individual types). Their thermal degradation leads in both cases to the same products. From all the above mentioned facts it follows that the difference is either in the configuration at $C_{(9)}$ or at $C_{(10)}$. Three examples of isomers at $C_{(10)}$ were already described in the studied groups of compounds: the aglycone aklavinone-I (*III*), (ref.[7]) and two glycosides collinemycin and mimimycin[8,9] that have an aglycon *IV*, isomeric with ε-pyrromycinone (*V*), which is 1-hydroxyanalog of aklavinone (*VI*). No $C_{(9)}$-epimers were found in the nature; some analogs of daunomycinone were synthetized[10–13]. With respect to the ^{1}H-NMR spectrum of *I* is the signal of $COOCH_3$ in the spectrum of our compound shifted downfield and those of $H_{(10)}$ and side chain methyl are



	R^1	R^2	R^3	R^4
I	H	H	$COOCH_3$	H
III	H	OH	H	$COOCH_3$
IV	OH	OH	H	$COOCH_3$
V	OH	OH	$COOCH_3$	H
VI	H	OH	$COOCH_3$	H
VII	H	H	H	$COOCH_3$
X	OH	H	$COOCH_3$	H

shifted upfield (Table I). Same trend was observed with signals of those protons in collinemycin and mimimycin[8] when compared with the corresponding glycosides of the ε-pyrromycinone series and also by comparison of the spectra of aklavinone

TABLE I

^{1}H-NMR Chemical shifts of selected protons in compounds *I*, *II* and *VII*

Compound	$H_{(7)}$, $H_{(8)}$	$H_{(10)}$	$COOCH_3$	$H_{(13)}$	$H_{(14)}$
I[a]	2·20—3·10[b]	3·95 s	3·73 s	1·65 q	1·08 t
II[a]	2·69—3·14[b]	3·90 s[c]	3·72 s	2·45 q	1·06 t
VII	2·35—3·12[b]	3·92 s	3·84 s	1·63 q	1·00 t

[a] Ref.[6]; [b] AA'BB'system, 4 H; [c] 2 H; Abbreviations: s singlet, d doublet, t triplet, q quartet.

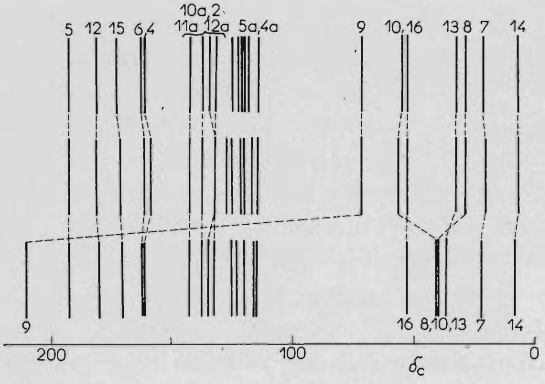


FIG. 1

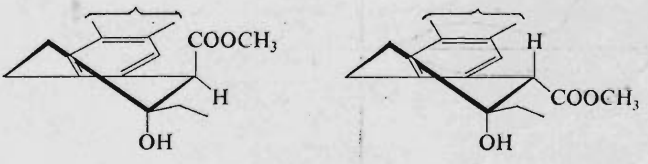
Schematic comparison of ^{13}C-NMR chemical shifts of compounds *I*, *II* and *VII*

TABLE II

Comparison of ^{13}C-NMR chemical shifts of two pairs of $C_{(10)}$-epimers (*I*, *V*, and *VII* in deuteriochloroform, *IV* in deuteriodichloromethane)

Atom	*V*[a]	*IV*[b]	*V*−*IV*	*I*[c]	*VII*	*I*−*VII*
1	158·7	157·6	1·1	119·8	119·9	−0·1
2	130·5	129·9	0·6	137·0	137·1	−0·1
3	130·8	129·5	1·3	124·5	124·5	0·0
4	159·3	158·2	1·1	160·9	160·8	0·1
5	191·6	190·2	1·4	192·7	193·0	−0·3
6	163·2	161·4	1·8	158·2	162·6	−3·4
7	71·3	70·9	0·4	19·9	21·3	−1·4
8	34·9	33·3	1·6	28·2	28·8	−0·6
9	71·7	71·9	−0·2	71·5	71·3	0·2
10	57·9	55·8	2·1	55·9	54·7	1·2
11	121·0	120·8	0·2	121·1	120·5	0·6
12	186·7	185·5	1·2	181·5	181·3	0·2
13	33·5	29·6	3·9	32·2	32·2	0·0
14	7·1	8·0	−0·9	6·6	7·3	−0·7
15	172·0	171·2	0·8	171·4	173·1	1·7
16	52·9	52·3	0·6	52·5	52·6	−0·1
4a	113·1	112·4	0·7	114·2	113·6	−0·6
5a	115·5	114·1	1·4	116·7	117·9	−1·2
6a	133·5	131·1	2·4	126·9	122·3	4·6
10a	143·5	142·6	0·9	141·9	142·4	0·5
11a	132·5	132·3	0·2	130·7	133·6	2·9
12a	113·3	112·2	1·1	130·7	130·9	0·2

[a] Aglycone part of marcellomycin[8] (ε-pyrromycinon, *V*); [b] aglycone part of mimimycin[8] (10-epi-ε-pyrromycinon, *IV*); [c] ref.[6].



I *VII*

FIG. 2

Assumed conformation of the alicyclic ring of compounds *I* and *VII*

(*VI*) and aklavinone-I (*III*), (ref.[7]). The assumption of half-chair conformation of the alicyclic ring (Fig. 2) in both cases allows an easy interpretation of the observed facts. The ester methyl in 7-deoxyaklavinone *I* is situated above the plane of the aromatic ring C and is therefore shielded owing to the ring currents whereas in its $C_{(10)}$-epimer *VII* is on the contrary more shielded the $H_{(10)}$-proton. The methyl of the ethyl group is with $C_{(10)}$-epimer more exposed to the anisotropic effect of the carbonyl group what explains its upfield shift. Also the differences between the ^{13}C-NMR spectra of *I* and our compound are similar to that found between the aglycone parts of the marcellomycin and mimimycin molecules[8] (Table II). Further arguments are provided by comparison of CD spectra. Similarly to the pair aklavinon–aklavinon-I (ref.[7]), is the CD curve of compound *VII* in the region of 270–390 nm a mirror image that of *I* (Fig. 3). 1,5-Diazabicyclo[4.3.0]non-5-ene (DBN) which was used

TABLE III

Semiquantitative evaluation of products of thermal degradation of 7-deoxyaklavinone (*I*) based on TLC and HPLC

t, °C	*I*	*II*	*VIII*	*IX*
70	+++	–	–	–
100	+++	traces	traces	traces
145	+++	+	+[a]	+
185	traces	++	+[a]	++

[a] Ratio *VIII* : *IX* was determined on the basis of ^{1}H-NMR spectrum integration in the region of phenolic hydroxyls. It was 1 : 4 and 1 : 2·8 at 145°C and 185°C, respectively.

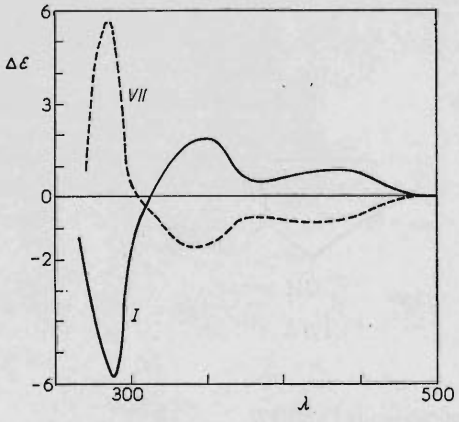
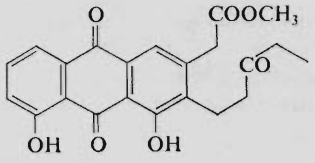


FIG. 3

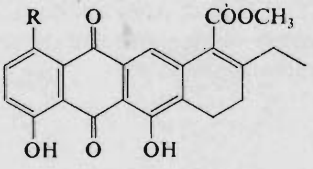
Circular dichroism curves of compounds *I* and *VII*

for the conversion of collinemycin to musettamycin and mimimycin to marcellomycin[8], also catalyzed the isomerization of *I* to our compound (ratio *I* : *VII* was 1·50 : 1) and *vice versa* (*I* : *VII* = 1·37 : 1). From the above mentioned facts it follows that the compound under discussion has the formula *VII* and the structure of (9*R*, 10*S*)-7-deoxyaklavinone. Predominance of *I* in the isomerization mixtures shows that the "natural" (9*R*,10*R*)-7-deoxyaklavinone is thermodynamicaly more stable.

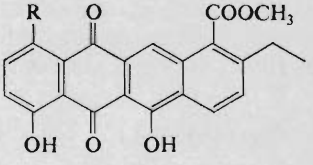


II

Similarly to the above mentioned $C_{(10)}$-epimers, a question arises whether compounds *II* and *VII* are true natural compounds. We tried to solve this problem by investigating the behaviour of *I* at pH and temperature changes. We found using TLC and HPLC that 7-deoxyaklavinone (*I*) does not change upon three hours' standing in 10% HCl at room temperature. In 1M-NaOH under similar conditions, a certain amount of more polar compounds is formed but the reaction mixture does not contain neither *II* or *VII*. Thermic decomposition of *I* and *VII* produces mixtures of similar composition. Besides the dehydration leading the *VIII*, the compound *II* is formed by retroaldolization and the dehydration plus dehydrogenation yields bisanhydroaklavinone *IX*. Experiments summarized in Table III show that at 70°C no reaction takes place, around 100°C traces of *II*, *VIII* and *IX* are formed and the decomposition is complete above 180°C. At lower temperatures, more *VIII* than *IX* is formed. This ratio is reversed by increase of temperature. Brockmann[9] reported the formation of bisanhydro derivative *XI* from 7-deoxypyrromycinone (*X*) on Pd/C but not on dehydration. Our results agree with his observations concerning the dehydration of *X* but we found that lower temperature is sufficient.



VIII, R = H
XII, R = OH



IX, R = H
XI, R = OH

Because in our isolation procedure[6] no conditions enabling formation of *II* or *VII* were used, both compounds are natural. Isolation of *VII* means that $C_{(10)}$-epimers

exist at all three possible levels: in glycosides (anthracyclines), aglycones (anthracyclinones) and 7-deoxyaglycones. That helps to understand the richness of the mixture of metabolites in the *Streptomyces* strains under discussion. Simultaneous presence of compounds belonging to both series of epimers points out to the relative stereospecifity of the cyclization (eventually epimerization) reaction only.

EXPERIMENTAL

Melting points were determined in a Kofler apparatus. UV/VIS spectra were measured in cyclohexane on a Cary 118 C spectrophotometer. CD spectra were measured in dioxan using a Rousset-Jouan CD 185 Dichrograph instrument. Infrared spectra were recorded in KBr pellets on a Unicam SP-200 spectrophotometer. Mass spectra were studied by a Varian MAT 311 mass spectrometer; ion source temperature 200°C, direct inlet at 150°C, energy of ionizing electrons 70 eV, ionizing current 1 mA. Elemental composition of ions was determined by peak-matching technique using perfluorokerosene as an standard (accuracy ± 5 ppm). ^{1}H-NMR spectra were measured on a Jeol FX-60 spectrometer (59·797 and 15·036 MHz, FT mode) at 25°C in deuteriochloroform containing tetramethylsilane as an internal standard. Chemical shifts were calculated with accuracy $\pm 0{\cdot}005$ and $\pm 0{\cdot}06$ ppm from the digitally obtained address differences. Signal assignments given in Table II are based on the off-resonance and decoupling experiments complemented by spectra comparison between related compounds. HPLC determinations were made on a Spectra Physics SP 8000 liquid chromatograph: column Separon SO VSK, 250 × 4 mm, system n-hexane–chloroform–methanol 52 : 45 : 3 (S1), flow rate 0·5—1·0 mol/min, temperature 22 °C, UV detection at 270 nm. Silufol R^{20} (Kavalier, Votice, Czechoslovakia) was used both for preparative and analytical TLC in the system n-heptane–chloroform–methanol 40 : 40 : 10 (S2). The R_F values for *I*, *VII*, *VIII* and *IX* were 0·41, 0·42, 0·71 and 0·71.

Isolation of (9*R*,10*S*)-7-deoxyaklavinone *VII*

Procedure described earlier[6] was adopted. Mother liquors after crystallization of *I* (48 mg) were evaporated and subjected to preparative chromatography in the system S2. Four times elution of the plate by this solvent mixture yielded 27 mg of *I* and 15 mg of *VII*, m.p. 159—161°C (ethanol).

Isomerization *I*→*VII*

The agens (2 µl 1,5-diazobicyclo[4.3.0]non-5-ene) was added to the solution of *I* (0·8 mg) in 0·2 ml dichloromethane and the mixture was allowed to stand 24 h at 22°C. After acidification to pH 4 by tartaric acid, it was extracted by chloroform, the solvent was removed and the residue was analyzed by HPLC. Same procedure was applied to the epimerization of *VII*.

Thermal Degradation of *I*

7-Deoxyaklavinone *I* (75 mg) was dissolved in chloroform and 0·5 g of silica gel was added. The solvent was removed and the residue was heated 3 h at 185°C, cooled, extracted by chloroform and subjected to preparative chromatography on Silufol R^{20} in system S2. First fraction contained 7 mg of *I*, second 26 mg of *II*, and third (32 mg) was a mixture of *VIII* and *IX*. Five times repeated crystallization from ethanol yielded 7 mg of compound *IX*, m.p. 227°C. Mother liquors were evaporated, dissolved in chloroform and precipitated by light petroleum. The

crystallization from chloroform–methanol mixture yielded 4 mg of *VIII*, m.p. 173—174°C. (9*R*,10*S*)-7-Deoxyaklavinone (*VII*), yellow crystals m.p. 159—161°C. UV/VIS spectra λ_{max} 259, 290, 432, 452 nm.

Mass spectrum *m/z* (% of relative intensity, composition): 396 (2, $C_{22}H_{20}O_7$), 378 (17, $C_{22}H_{18}O_6$), 349 (7, $C_{20}H_{13}O_6$), 340 (7, $C_{19}H_{16}O_6$), 319 (100, $C_{20}H_{15}O_4$), 307 (32, $C_{18}H_{11}O_5$), 279 (31, $C_{17}H_{11}O_4$), 265 (8, $C_{16}H_9O_4$).

9-*Ethyl*-4,6-*dihydroxy*-10-*carbmethoxy*-7,8-*dihydro*-5,12-*naphthacenequinone* (VIII). Yellow needles, m.p. 173—174°C, optically inactive. UV/VIS spectra λ_{max} 253, 255, 287, 295, 440, 454.

Mass spectrum: 378 (81, $C_{22}H_{18}O_6$), 376 (19, $C_{22}H_{16}O_6$), 361 (17, $C_{21}H_{18}O_6$), 346 (100, $C_{21}H_{14}O_5$), 318 (43, $C_{20}H_{14}O_4$), 303 (20, $C_{19}H_{11}O_4$), 290 (25, $C_{18}H_{10}O_4$). ^{1}H-NMR spectrum: 1·16 t (J = 7·3 Hz, 3 H), 2·43 q (J = 7·3 Hz, 2 H), 2·32—3·08 AA″BB″ (4 H), 3·94 s (3 H), 7·20—7·88 mt (3 H), 12·13 s (1 H), 12·37 s (1 H).

REFERENCES

1. Vaněk Z., Tax J., Komersová I., Sedmera P., Vokoun J.: Folia Microbiol. (Prague) *22*, 139 (1977).
2. Takahashi Y., Naganawa H., Takeuchi T., Umezawa H., Komiyama T., Oki T., Inui T.: J. Antibiot. *30*, 619 (1977).
3. Oki T., Shibamoto N., Matsuzawa Y., Ogasawara T., Yoshimoto A., Kitamura I., Inui T., Naganawa H., Takeuchi T., Umezawa H.: J. Antibiot. *30*, 683 (1977).
4. Bachur N. R.: Cancer Treat. Rept. *63*, 817 (1979).
5. Komiyama T., Oki T., Inui T.: J. Antibiot. *32*, 1219 (1979).
6. Jizba J. V., Sedmera P., Vokoun J., Blumauerová M., Vaněk Z.: This Journal *45*, 764 (1980).
7. Tresselt D., Eckardt K., Tax J.: Tetrahedron *31*, 613 (1975).
8. Doyle T. W., Nettleton D. E., Grulich R. E., Balitz D. M., Johnson D. L., Vulcano A. L.: J. Amer. Chem. Soc. *101*, 7041 (1979).
9. Brockmann H.: Fortschr. Chem. Org. Naturst. *21*, 122 (1963).
10. Wong C. M., Popien D., Schwenk R., Te Raa J.: Can. J. Chem. *49*, 2722 (1971).
11. Arcamone F., Bernardi L., Giardino P., Patelli B., Di Marco A., Casazza A. M., Pralesi G., Reggioni P.: Cancer Treat. Rept. *60*, 829 (1976).
12. Kende A. S., Curran D. P., Tsay Y., Mills J. E.: Tetrahedron Lett. *1977*, 3537.
13. Kelly T. R., Tsang W. G.: Tetrahedron Lett. *1978*, 4457.

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