Isolation of Maridomycins and Structure of Maridomycin II

Maridomycins, a new group of macrolide antibiotics, were obtained from *Streptomyces hygroscopicus*[1], and named as maridomycin I, II, III, IV, V and VI, respectively, and characterized as follows:

All of these antibiotics show nothing but ultraviolet end absorption in methanol. They are classified as macrolide antibiotics from their physico-chemical, chemical and microbiological properties. The structure of maridomycin II was elucidated as shown in the chart.

Maridomycin II (II) was obtained as colorless prisms, pKa' 6.9, IR[2]: 1740 (−O−CO−), 1235 (−OAc), 2730 (−CHO), NMR[3]: 1.01 (9H, *d*, −CH−$(CH_3)_2$, −CH−CH_3), 2.25 (3H, *s*, −O*Ac*), 2.54 (6H, *s*, −N$(CH_3)_2$), 3.56 (3H, *s*, −OCH_3), 5.66 (1H, *dd*, >C=C<), 6.10 (1H, *dd*, >C=C<), 9.65 (1H, *s*, −*CH*O); (in d_6-Me_2CO), 3.96 (1H, *q*, HO−C−*H*, J = 9, 2.5 Hz), 6.04 (1H, *dd*, >C=C<, *J* = 16, 9 Hz), 5.49 (1H, *dd*, >C=C<, *J* = 16, 9 Hz), 3.10 (1H, *dd*?, −O−*H*). NMR inspection and spin decoupling experiments of II showed that the compound contains HO−C(H)(H)−C(H)=C(H)−C(H)(O)−C(H)(H)− group.

When II was acetylated with one mole of acetic anhydride, the 2′-monoacetate (VIII), $C_{44}H_{71}NO_{17}$, pKa' 4.7, MS: *m/e* 885 (M^+), NMR: 2.06 (3H, *s*, −OAc), 4.02 (1H, *q*, HO−C−*H*) was obtained. An alternative acetylation of II with one mole of acetyl chloride gave the 9-monoacetate (IX), pKa' 6.6, MS: *m/e* 885 (M^+).

Acetylation of VIII and IX led to the same diacetate $C_{46}H_{73}NO_{18}$ (VII), $[\alpha]_D^{29}$ −81.4° (c = 0.5 in EtOH), pKa' 4.7, MS: *m/e* 927 (M^+), IR ($CHCl_3$): 3480 (−C−OH), 1240 (−OAc), NMR: 2.02, 2.04 (each 3H, *s*, −OAc).

On catalytic hydrogenation II gave tetrahydro II (X), $C_{42}H_{73}NO_{16}$, NMR: disappearance of olefinic protons of II at 5.5–6.3 ppm, and on acetylation X afforded the triacetate (XI), $C_{48}H_{79}NO_{19}$, IR ($CHCl_3$): 3500 (−C−OH), 1240 (−OAc), NMR: 2.00, 2.02, 2.06 (each 3H, *s*, −OAc).

Mild acid hydrolysis (0.05 *N* · HCl) of II yielded (XII), $C_{42}H_{71}NO_{17}$, and acetylation of XII gave the tetraacetate (XIII), $C_{50}H_{79}NO_{21}$, NMR: 2.02, 2.06 (each 6H, *s*, −OAc), 5.6–6.1 (2H, *m*, >C=C<). On catalytic hydrogenation, XII took up one molar equivalent of hydrogen and the dihydro XII (XIV), $C_{42}H_{73}NO_{17}$, NMR: disappearance of olefinic protons, was obtained.

The existence of an aldehyde group in II was suggested from its NMR and was ascertained from the formation of the alcohol (XXIV) by $NaBH_4$ reduction, and the thiosemicarbazone (XXV), UV: λ_{max}^{EtOH} 270 nm, IR: 1595 (NH_2), 1520 (−C(=S)−N), NMR: 7.44 (1H, *t*, −CH_2−*CH*=N−).

Hydrolysis of II with 1 N KOH afforded one mole each of acetic and isovaleric acids. Hydrolysis of II with 0.5 *N*·HCl gave a lipophilic neutral sugar which was identified as 4−*O*−isovaleryl mycarose[4] (XVII) by comparison with authentic sample obtained from leucomycin A_3.

Methanolysis of II with MeOH−HCl yielded neutral sugars, which were further separated into α-methyl 4-*O*-isovaleryl-L-mycaroside[5] (XIX), MS: *m/e* 260 (M^+), $[\alpha]_D^{21}$ −151° (c = 1.0 in $CHCl_3$) and β-methyl 4-*O*-isovaleryl-L-mycaroside[5] (XVIII), MS: *m/e* 260 (M^+), $[\alpha]_D^{21}$ +8.6° (c = 1.5 in $CHCl_3$). Both compounds were identified with authentic samples obtained from leucomycin A_3.

Acid hydrolysis of (X) under mild conditions gave demycarosyl tetrahydro II (XX), $C_{30}H_{53}NO_{12}$, $[\alpha]_D$ −19.9° (c = 1.0 in EtOH), NMR: 2.30 (3H, *s*, OAc), 2.50 (6H, *s*, −N$(CH_3)_2$), 3.55 (3H, *s*, −OCH_3), 3.90 (1H, *q*, −O−C−*H*), 4.44 (1H, *d*, $H_1'_a$, J = 7Hz), 5.35 (1H, *m*, AcO−C_3−*H*), 9.62 (1H, *s*, −*CH*O).

Acetylation of XX afforded tetraacetyl demycarosyltetrahydro II (XXI), $C_{38}H_{61}NO_{16}$, MS: *m/e* 787 (M^+), NMR: 2.01 (9H, *s*, OAc), 2.04, (3H, *s*, OAc), IR ($CHCl_3$): absence of OH group.

[1] Detailed microbiological and isolation studies will be published in J. Antibiotics (Tokyo).

[2] MW, molecular weight; VPO, vapour pressure osmometry in ethyl acetate; MS, mass spectrum; MIC, minimum inhibitory concentration against *Staphylococcus aureus* (mcg/ml); NMR, nuclear magnetic resonance spectrum in $CDCl_3$ (δ (ppm)); IR, infrared spectrum (KBr, cm^{-1}); UV, ultraviolet spectrum; TLC, thin layer chromatography (SiO_2).

[3] *s* = singlet, *d* = doublet; *dd* = double doublet; *t* = triplet; *q* = quartet; *oct* = octet; *m* = multiplet; J = coupling constant.

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Name		m.p. (decomp.)	$[\alpha]_D^{23}$ (c = 1.0 in EtOH)	MW[2] (VPO in EtOAc)	MS[2] *m/e* M^+	Mol. Formula	MIC[2] (mcg/ml)
Maridomycin I	(I)	129–132°	−72.3°	910	857	$C_{43}H_{71}NO_{16}$	0.5
Maridomycin II	(II)	134–136°	−71.9°	881	843	$C_{42}H_{69}NO_{16}$	0.5
Maridomycin III	(III)	135–138°	−76.0°	911	829	$C_{41}H_{67}NO_{16}$	1.0
Maridomycin IV	(IV)	143–146°	−76.2°	896	815	$C_{40}H_{65}NO_{16}$	2.0
Maridomycin V	(V)	144–149°	−73.6°	882	815	$C_{40}H_{65}NO_{16}$	5.0
Maridomycin VI	(VI)	149–154°	−77.7°	864	801	$C_{39}H_{63}NO_{16}$	5.0

Acid hydrolysis under vigorous condition ($2N \cdot HCl$, reflux) of XX gave an aminosugar, mycaminose[6] (XXII), m.p. 113–115°, which was identical with an authentic sample obtained from leucomycin A_3.

Maridomycin II (II) was oxidized with CrO_3-pyridine complex or MnO_2 to dehydromaridomycin II (XXVI), m.p. 206–207 °C (decomp.), which was identical with authentic sample of carbomycin[7] in UV-, IR-, MS-, NMR-spectra, specific rotation and Rf values on TLC.

The structure of carbomycin has been elucidated by WOODWARD et al.[8].

From these findings, the structure of maridomycin II was determined to be II. Its absolute configuration, except for the C_9-hydroxyl group, was also clarified. Further treatment of XXVI with KI in AcOH yielded dehydrodeepoxymaridomycin II which was identical with carbomycin B[7] in all respects.

Zusammenfassung. Das aus *Streptomyces hygroscopicus* isolierte neue Makrolid Maridomycin II lässt sich mit Säure Mycarcose und Mycaminose spalten. Auf Grund der Oxidation ins Carbomycin sowie der spektroskopischen Daten wurde die Struktur als II erklärt.

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Effect of Diurnal Rhythm and Food Withdrawal on Serum Lipid Levels in the Rat

Occasionally, we have observed from one day to the next marked differences in the levels of serum triglycerides of normal rats. Since fasting can affect serum and liver lipid levels[1,2] and thus may obscure any changes caused by a given treatment, our animals had been allowed access to food until they were killed. When rats of similar age, weight and body weight gain were used, the within group variation of serum triglycerides in both treated and untreated animals rarely exceeded 15%.

BARRETT[3] has found that plasma free fatty acids of rats exhibit a marked diurnal rhythm. This is in accord with the finding of extremely high turnover rates of free fatty acids in the plasma[4]. As far as we are aware, no studies have been conducted on the effect of diurnal rhythm on serum triglycerides in the fed and fasted rat. Therefore, experiments were carried out to determine the effect of fasting and killing time on rat serum triglycerides (as well as on cholesterol and phospholipids).

Methods. Since hemolyzed blood was sometimes obtained in our studies, we first determined the effect of hemolysis on serum triglycerides. Unhemolyzed blood was collected from untreated fed albino rats (Charles River) at 09.00 h and each sample was immediately divided into 2 tubes. The blood from one group was allowed to stand for 3 h and clear serum was obtained after centrifugation. The blood from the second group was hemolyzed with the aid of wooden applicators and centrifuged. Serum triglyceride levels were measured by the semi-automated method of KRAML and COSYNS[5].

For the studies on diurnal rhythm, male albino rats, weighing 180–190 g, were fed Purina lab chow ad libitum. Lighting was automatically regulated to provide 12 daily h of light from 08.00 to 20.00 h. Animals were kept under observation for 3 days and only those with normal weight gain and food intake were used. On day of killing, food (but not water) was withdrawn from half the animals at 08.00 h and the rats were decapitated at various times during the next 24 h. Serum triglycerides[5], cholesterol[6], and phospholipids[7] were measured according to previously described techniques.

Results and discussion. The results of the hemolysis study are presented in the Table. It was found that hemolysis had no effect on serum triglyceride levels in both male and female rats.

The effect of fasting and diurnal rhythm on serum lipids is presented in the accompanying Figure. It was found that both fasting and killing time had a profound effect on serum triglyceride levels. In fed rats, triglyceride glycerol levels at 08.00 h were 14 mg/100 ml and declined to a minimum of 7–8 mg/100 ml in the early evening. Fasting levels were markedly lower than those of fed rats, reaching a minimum of 2–3 mg/100 ml in the evening and

Effect of hemolysis on rat serum trigliyceride levels

Serum	Triglyceride glycerol (mg/100 ml)	
	Male rats	Female rats
Unhemolyzed	15.5 ± 1.43	10.6 ± 1.10
Hemolyzed	16.0 ± 1.64	10.0 ± 0.82
Mean difference	0.5 ± 0.92	0.6 ± 1.12

Blood from Charles River albino rats, weighing 180–190 g was used. Mean difference refers to difference in glycerol levels for each animal as a result of hemolysis. Results are presented as mean ± standard error for 10 rats/group.

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