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Note

Analysis of the macrolide antibiotics propionylmaridomycins and related compounds by reversed-phase high-performance liquid chromatography

G. A. BENS*, W. VAN DEN BOSSCHE, G. VAN DER WEKEN and P. DE MOERLOOSE
Department of Pharmaceutical Chemistry and Drug Quality Control, State University of Ghent, Faculty of Pharmaceutical Sciences, Harelbekestraat 72, B-9000 Ghent (Belgium)
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Propionylmaridomycins (PMDMs) are sixteen-membered ring macrolide antibiotics, belonging to the leucomycin group[1]. They were derived from maridomycins (MDMs)[2–4] by selective acylation with propionylchloride. In contrast to other members of the leucomycin group, such as spiramycins, turimycins and leucomycins, PMDMs and MDMs do not show a UV absorption maximum at 232 nm, but an end-absorption maximum at around 200 nm due to the lack of a conjugated diene bond (Table I).

PMDMs and MDMs have been separated on silica gel by a time-consuming high-performance liquid chromatographic (HPLC) procedure with refractometric detection[5] and by thin-layer chromatography on silica gel layers combined with an iodine reaction and subsequent determination of the iodine in the scraped-off silica gel[6].

In previous papers, we described chromatographic systems for separating spiramycins and turimycins[7–9] and it seemed obvious that these systems could also separate PMDMs and MDMs. As it was our intention to use UV photometric detection instead of refractometric detection, only the reversed-phase system described here was selected, as the eluents used in HPLC on silica gel showed a UV cut-off at around 220 nm.

EXPERIMENTAL

High-performance liquid chromatography

A Waters M-6000A pump (Waters Assoc., Milford, MA, U.S.A.) was used, fitted to a variable-wavelength detector (Varichrom; Varian, Palo Alto, CA, U.S.A.) at a wavelength setting of 203 nm and with nitrogen flushing through the lamp compartment.

Separations were carried out on an RSil-C_{18}-HL packing of 5-μm particles (Alltech Europe, Eke, Belgium), which is a microparticulate chemically bonded octadecylsilane reversed-phase packing with 18% bonded organic material. Home-made columns (100 × 3.2 mm I.D.) were filled by a slurry technique with methanol–carbon tetrachloride (10:90) as the suspending medium and acetonitrile–water (50:50) as the pressurizing eluent.

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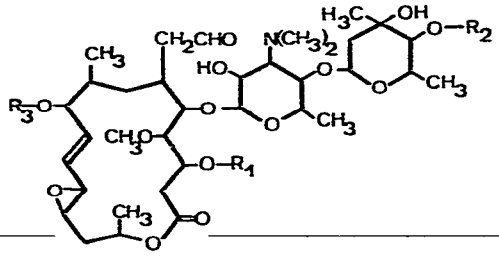
TABLE I

STRUCTURES OF PROPIONYLMARIDOMYCINS AND MARIDOMYCINS



Compound	*No.*	R_1	R_2	R_3
Propionylmaridomycin	1	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$	$COCH_2CH_3$
	2	$COCH_3$	$COCH_2CH(CH_3)_2$	$COCH_2CH_3$
	3	$COCH_2CH_3$	$COCH_2CH_3$	$COCH_2CH_3$
	4	$COCH_3$	$COCH_2CH_3$	$COCH_2CH_3$
	5	$COCH_2CH_3$	$COCH_3$	$COCH_2CH_3$
	6	$COCH_3$	$COCH_3$	$COCH_2CH_3$
Maridomycin	1	$COCH_2CH_3$	$COCH_2CH(CH_3)_2$	H
	2	$COCH_3$	$COCH_2CH(CH_3)_2$	H
	3	$COCH_2CH_3$	$COCH_2CH_3$	H
	4	$COCH_3$	$COCH_2CH_3$	H
	5	$COCH_2CH_3$	$COCH_3$	H
	6	$COCH_3$	$COCH_3$	H

Materials and reagents

Acetonitrile was of HPLC grade (Baker, Deventer, The Netherlands). Doubly distilled water was purified from UV-absorbing substances by pumping it through a Lobar RP-8 semi-preparative reversed-phase column (Merck, Darmstadt, G.F.R.). Diethylamine was of analytical-reagent grade (UCB, Brussels, Belgium) but was re-distilled *ex tempore* in an all-glass apparatus.

MDMs and PMDMs as pure compounds and bulk powder were obtained from Takeda Chemical Industries (Osaka, Japan). Pure spiramycins were obtained from Specia (Paris, France).

Analytical procedure for PMDM determinations

Standard solutions containing 0.320–1.920 $mg \cdot ml^{-1}$ of PMDM 3, 0.060–0.360 $mg \cdot ml^{-1}$ of PMDM 4, 0.020–1.120 $mg \cdot ml^{-1}$ of PMDM 5, 0.042 $mg \cdot ml^{-1}$ of spiramycin 3 and 0.003 $mg \cdot ml^{-1}$ of spiramycin 2 (internal standards) were prepared in acetonitrile–water (1:1).

Solutions containing 1.2 $mg \cdot ml^{-1}$ of PMDM bulk powder, 0.042 $mg \cdot ml^{-1}$ of spiramycin 3 and 0.003 $mg \cdot ml^{-1}$ of spiramycin 2 were prepared with the same solvent mixture.

RESULTS AND DISCUSSION

To detect PMDMs and MDMs, the reagents used in the eluent had to be free

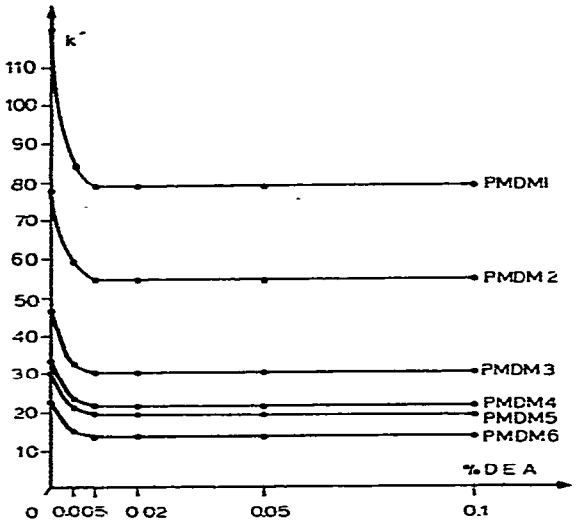


Fig. 1. k' values of propionylmaridomycins as a function of the percentage of diethylamine (DEA) in the eluent.

from UV-absorbing substances. The reversed-phase eluent acetonitrile–water–diethylamine (500:500:1) used for the chromatography of spiramycins and turimycins was suitable when the reagents as described under Materials and reagents were used. By this means an eluent was obtained that exhibited a transmission of more than 70%

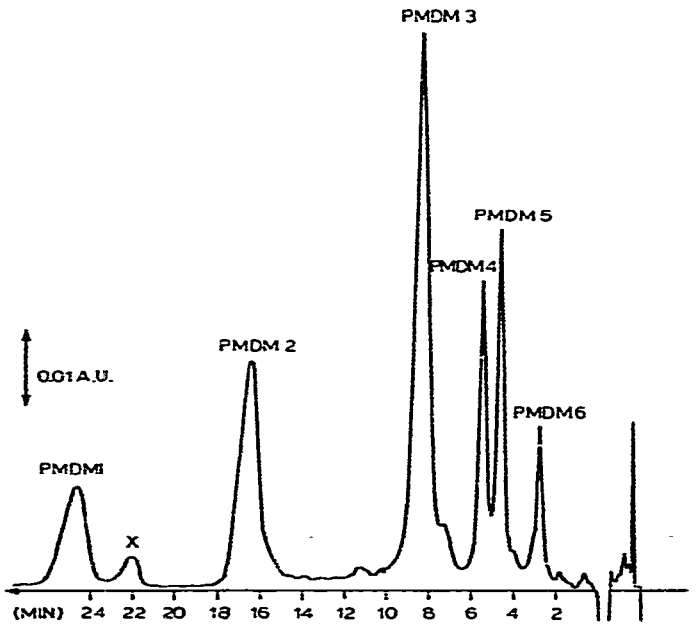


Fig. 2. Separation of propionylmaridomycins (1–6) (X = unknown compounds). Column: RSil-C18-HL, 5 μm, 100 × 3.2 mm I.D. Eluent: acetonitrile–water–diethylamine (500:500:0.1). Column temperature: 25°C. Flow-rate: 1.0 ml·min^{-1}. Detection: UV, 203 nm (under nitrogen).

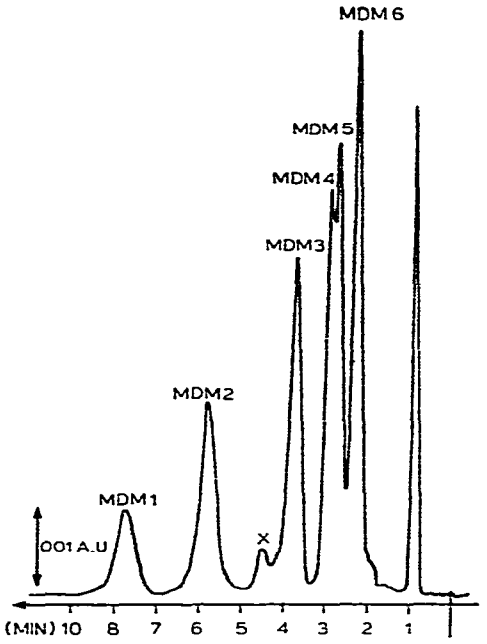


Fig. 3. Separation of maridomycins. Conditions as in Fig. 2.

at 203 nm, so PMDMs and MDMs could be detected. To avoid any influence of air-oxygen, nitrogen had to be blown through the lamp compartment of the detector. Propionylmaridomycins and maridomycins could be separated on a C_{18} reversed-phase column with the eluent described, but as the concentration of redistilled diethylamine was the limiting factor in obtaining an eluent with a very low UV cut-off, the effect of diethylamine concentration in the mobile phase was investigated.

As can be seen in Fig. 1, a concentration of 0.01% of diethylamine gave practically the same capacity ratios (k') for the different substances as a concentration of 0.1%, without loss of resolution. Therefore an eluent was used consisting of ac-

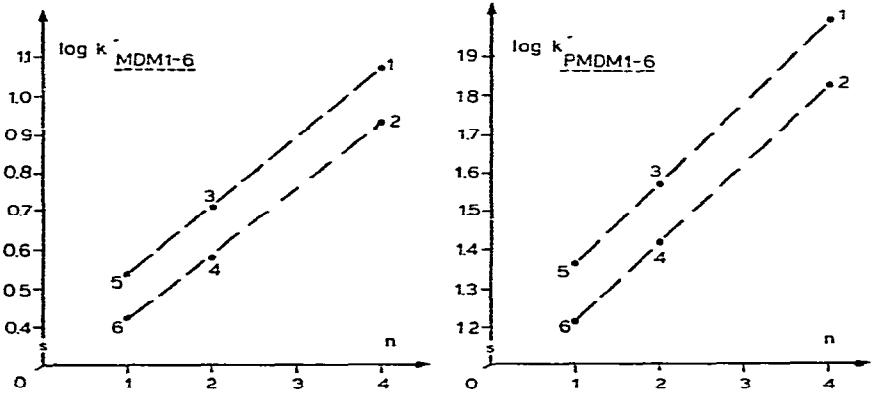


Fig. 4. Log k' *versus* n (n = number of alkyl carbon atoms in R_2) for the two homologous series of propionylmaridomycins (1, 3 and 5 and 2, 4 and 6) and maridomycins (1, 3 and 5 and 2, 4 and 6).

etonitrile–water–diethylamine (500:500:0.1). The chromatograms in Figs. 2 and 3 show the separations of propionylmaridomycins and maridomycins.

With their additional propionyl function, PMDMs are more apolar than the corresponding MDMs, resulting in higher k' values and increased elution times. The resolution between the isomers MDM 4 and MDM 5 could be improved by increasing the water content in the eluent, but this also resulted in increased k' values.

As the PMDM and MDM complexes each consist of two groups of homologues (*i.e.*, MDMs or PMDMs 1, 3 and 5 and 2, 4 and 6) a linear relationship was found between the logarithm of the capacity factors and the number of alkyl carbon atoms (n) in the acyl function at position 4″ (Fig. 4). The same relationship was also seen for the two groups of homologues in the leucomycin and turimycin complexes (*i.e.*, A_9, A_7, A_5 and A_1, identical with turimycins H_2, H_3, H_4 and H_5 and A_8, A_6, A_4 and A_3). The correlation coefficients for the linear regressions were always greater than 0.999. This result may be useful for the identification of unknown peaks in these groups or in other macrolide complexes.

In analytical studies of macrolide antibiotics, internal standards have seldom been used and, when they were[5,10], they did not have analogous structures. The main reason is that most of the macrolide antibiotics are complex mixtures and that pure components can be obtained in amounts of only a few milligrams. As the structurally analogous spiramycins (Sp) were available in sufficient amounts in a pure state, they were tested as a possible internal standard in the determination of PMDMs in a bulk powder. This powder contained PMDM 3 as a major component and minor amounts of PMDM 4 and PMDM 5. As the PMDM 3 concentration was about 18 times that of the two other components, two internal standards were chosen (spiramycin 3 for PMDM 3 determination and spiramycin 2 for PMDM 4 and 5 determination). Fig. 5 shows the separation of these propionylmaridomycins and spiramycins.

Standard solutions and solutions of the PMDM bulk powder were injected on the column in triplicate by means of a six-way valve with a fixed loop of 20 µl. Concentrations of PMDMs 3, 4 and 5 were calculated from calibration graphs, which were constructed by plotting peak-height ratios of PMDM 3 to spiramycin 3, PMDM 4 to spiramycin 2 and PMDM 5 to spiramycin 2 against the concentrations of PMDM 3, 4 and 5, respectively, in the standard solutions.

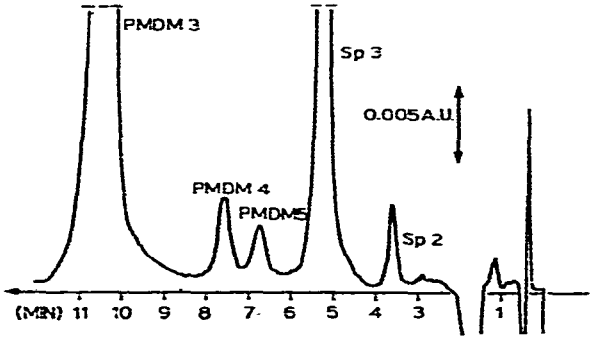


Fig. 5. Separation of propionylmaridomycins 3, 4 and 5 present in a typical bulk powder and the internal standards spiramycins 2 and 3 (Sp 2 and Sp 3). Conditions as in Fig. 2 except flow-rate, 0.8 ml·min^{-1}.

The graphs were rectilinear ($r > 0.998$), although large concentrations were injected on the analytical column. This procedure indicated that the PMDM bulk powder contained 89.25% of PMDM 3 (±1.51% coefficient of variation, C.V.), 6.35% of PMDM 4 (±2.25% C.V.) and 4.33% of PMDM 5 (±5.2% C.V.) ($n = 5$).

CONCLUSION

With the eluent system described it is possible to separate propionylmaridomycins and maridomycins by reversed-phase HPLC. It also permits the precise determination of the components of a typical propionylmaridomycin bulk powder faster than previously described and with UV detection instead of refractometric detection.

The linear relationship between the logarithms of the capacity factors and the number of alkyl carbon atoms in the acyl function in position 4″ for the two groups of homologues in MDM and PMDM mixtures may contribute in the identification of unknown peaks in these and in similar macrolide antibiotic complexes.

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