BIOCONVERSION OF MONENSIN BY A SOIL BACTERIUM, *SEBEKIA BENIHANA*

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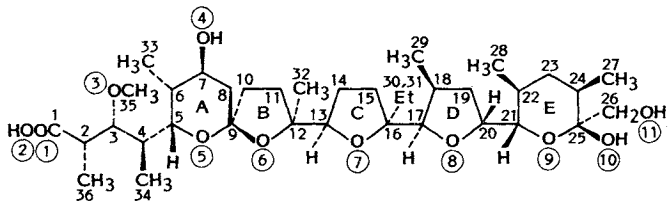
(Received for publication February 26, 1990)

Monensin (**1**), a carboxylic polyether antibiotic has been extensively used as an anticoccidial agent for poultry[1] and to improve the efficiency of feed utilization in ruminant animals[2]. Despite the considerable economic and environmental implications of this extensive use in animals, few data are available on the detoxification processes of such molecules. We described recently the bioconversion of nigericin[3] by *Sebekia benihana* NRRL 11111[4]. This soil bacterium was found to convert nigericin in three successive steps, giving compounds which had no ionophoric and antibiotic properties.

S. benihana also proved to be efficient on monensin, which was converted to three major compounds in similar fashion. This paper deals with the determination of the structures of these three products, and of their antibiotic activity and ionophoric properties.

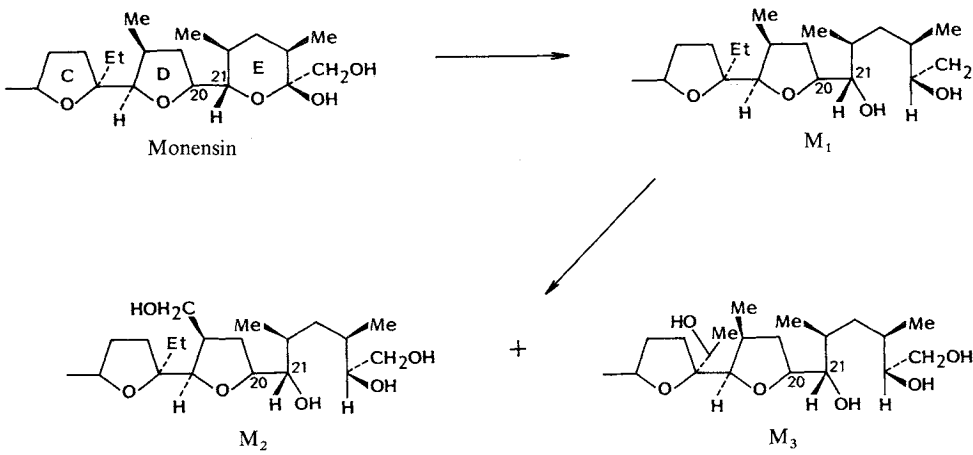
Monensin (40 mg in 2 ml ethanol solution) was bioconverted by whole cells of *S. benihana* NRRL 11111 grown in 100 ml TYG medium as previously described for nigericin[3], quantitatively into M_1 after 24 hours; M_1 was then oxidized to M_2 and M_3 after 120 hours (Fig. 1). The bioconversion products were detected and isolated as previously described[3]; their Rf values were as follow; monensin 0.84, M_1 0.56, M_2 0.26, M_3 0.20. (TLC: $CHCl_3$ - MeOH, 9 : 1).

The structures of M_1, M_2 and M_3 were identified by IR, FAB-MS and NMR spectroscopy[3]. Monensin, M_1, M_2 and M_3 have similar IR spectra indicating that the main skeleton of monensin is maintained. FAB (+) experiments confirmed the MW's of monensin derivatives as acid form $(M+H)^+$: M_1, 673.5; M_2, 689.6; M_3, 689.5). FAB (−) experiments confirmed the MW's of M_1 and M_2 ($(M-H)^-$: M_1, 671.3; M_2, 687.3). In conclusion, FAB-MS clearly shows the presence of an open terminal ring for M_1, M_2 and M_3 and an additional



Monensin (**1**)

Fig. 1. Bioconversion of monensin by *Sebekia benihana*.



oxidation for M_2 and M_3. The structures proposed for M_1, M_2 and M_3 were finally elucidated by NMR analysis. The general strategy followed for this purpose has been described previously[5]. ^{13}C and ^{1}H chemical shifts of M_1, M_2 and M_3 were compared with those of monensin. The main significant differences are reported in Tables 1 and 2. Omitted signals were very similar and in agreement with those described earlier for monensin (acid form)[6~8] suggesting that most of the monensin skeleton is unchanged, especially the carboxyl terminus of the molecule including the A and B rings. The NMR changes observed for M_1 and M_2 compared to monensin are very similar to those observed for bioconverted analogous products of nigericin (N_1 and N_2) compared to it[3]. For M_3 a new situation is encountered compared to the nigericin biotransformation: H_{3OA} and H_{3OB} (ring C) present in monensin disappear in favor of a single proton which is shifted to the CH(O) region; similarly C-30 is shifted to the same direction. Neighboring atoms (17 and 15) are also markedly affected. As previously observed for nigericin, the bioconversion of monensin consists of two types of reaction:

i) Reduction: The opening of the terminal ring results from an enzymatic reduction of the δ hydroxy-ketone which is in equilibrium with its hemiketal tautomeric form. A chemical reduction of monensin performed with $NaBH_4$ confirmed the presence of a reduced product. It led to two diasteroisomers M_1 (30%) and DM_1 (70%) that were purified by column chromatography on silica gel using a MeOH-$CHCl_3$ gradient (4~6.5% MeOH in $CHCl_3$). M_1 was eluted first (Rf: M_1, 0.56; DM_1, 0.47; TLC: $CHCl_3$-MeOH, 9:1). They could be identified by ^{13}C NMR and direct analogy with N_1 and DN_1 obtained from reduction of nigericin[3]. The same ^{13}C signals are shifted (C-25: M_1 76.58; DM_1, 73.7. C-27: M_1, 17.60; DM_1, 15.60). Consequently, the same stereochemistry of C-25 could be attributed to M_1 and N_1, *i.e.*, *S* configuration.

ii) Oxidation: M_2 is very similar to N_2[3], the same CH_3 is oxidized to CH_2OH, probably by a similar enzymatic process. The reaction does not continue to complete oxidation to COOH as observed for N_3. In the case of monensin, a new site of oxidation is found involving the ethyl group. These two oxidations proceed in parallel giving M_2 and M_3. The stereochemistry of the $-CHOH-CH_3$ remains to be solved. As M_3 is no longer an antibiotic (see later), no further investigations were performed.

The antibiotic properties of the bioconversion products M_1, M_2 and M_3 were evaluated classically by using the conventional dilution method with *Bacillus cereus* ATCC 14579 in Mueller-Hinton broth at pH 7. In spite of the opening of the terminal ring, M_1 is still an antibiotic with an MIC (3.12 μg/ml) only twice that of monensin (1.56 μg/ml). The situation is very different from that encountered for the bioconverted analogous product (N_1) whose MIC was fifty times higher that of nigericin. On the other hand, M_2 and M_3 are no longer antibiotics though their terminal ring is similar to M_1. Indeed, M_2 and M_3 have additional CH_2OH groups resulting from the oxidation process. As a consequence it is likely that the

Table 1. Comparison of ^{13}C chemical shifts (δ) of monensin and free acids of M_1, M_2 and M_3 in $CDCl_3$.

Carbon No.	Monensin	M_1	M_2	M_3
E ring				
25	**97.20**	76.58	76.88	76.82
26	68.00	64.31	64.48	64.46
27	16.40	17.60	17.52	17.67
24	35.80	34.85	34.52	34.94
23	36.80	38.54	37.98	39.23
22	32.90	34.16	34.68	34.42
21	74.00	77.41	77.94	77.90
D ring				
29	15.80	15.72	**62.05**	16.42
18	34.60	35.12	44.59	35.67
19	31.60	33.22	29.37	33.63
20	77.40	77.86	79.82	77.97
C ring				
30	31.20	30.30	25.30	**86.21**
31	8.60	8.19	7.49	18.17
15	32.70	30.90	31.86	28.37
(17)[a]	(85.10)	(85.48)	(83.48)	(71.11)

[a] Belongs to ring D but influenced by variation in ethyl group 30.

Table 2. Comparison of ^{1}H chemical shifts (δ) of monensin with M_1, M_2 and M_3 as free acids in $CDCl_3$.

Proton No.	Monensin	M_1	M_2	M_3
E ring				
25	**No signal**	3.49	3.50	3.51
24	1.49	1.80	1.99	1.75
23B	1.35	0.89	0.89	0.89
23A	1.50	1.85	1.92	2.01
D ring				
29	0.94	0.88	**3.94~3.79**	1.01
18	2.24	2.23	2.80	2.28
C ring				
30	1.57~1.57	1.46~1.46	1.72~1.55	**3.82**
31	0.97	0.90	0.92	1.20
15B	1.37	1.67	1.88	2.02

lipophilic external envelope of these molecules is too hydrophilic to be integrated in the cell membrane. The partition is in favor of the aqueous phase of the culture medium. This corresponds to a classical detoxification process of lipophilic compounds; it was already observed for nigericin[3] and grisorixin[9~11].

The complexation constants K_1 of monensin derivatives (expressed as their logarithm) for Na^+ and K^+ were determined by the classical method of extraction in a biphasic system[12], giving the following results; monensin Na^+: -3.80 ± 0.2, K^+: -4.92 ± 0.1; M_1 Na^+: -5.22 ± 0.2, K^+: -6.47 ± 0.1. Consequently M_1 still complexes with Na^+ and K^+ though less efficiently than monensin; the selectivity $Na^+ > K^+$ is maintained as well. We have shown previously[13] that N_1, in contrast, does not complex with K^+ and is no longer antibiotic. To explain these differences obviously linked to the structure of the bioconverted products M_1 and N_1, additional investigations are needed.

In conclusion this study of bioconversion by *S. benihana* affords interesting experimental results.

i) The detoxification process is clearly associated with the modification of the amphiphilic balance of the ionophores rather than with a change in the complexing site and shown from the difference between M_1 and N_1.

ii) It is possible to open the monensin terminal hemiketal ring without markedly upsetting the well organized complexing cavity of the natural metabolite.

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