# MOENOMYCIN. VII[1]

## ISOLATION AND PROPERTIES OF FURTHER COMPONENTS OF THE ANTIBIOTIC MOENOMYCIN

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Dedicated to Professor Dr. JOSEF SCHMIDT-THOMÉ on his 60th birthday anniversary.

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The new antibiotic, moenomycin, isolated from *Streptomyces bambergiensis* is a mixture of several, chemically very similar components, which are particularly characterized by their phosphorus content. The moenomycin complex can be separated into components A, $B_1$, $B_2$ and C by means of column chromatography on silica gel with *n*-propanol - 2N ammonia as eluant. The use of other chromatographic procedures, *e. g.* chromatography on anion exchange resins, results in a further separation of fraction $B_1$ into components D, E and F; similarly fraction $B_2$ can be separated into components G and H. Isolation and properties of moenomycins D, E, F, G and H are described.

The new antibiotic moenomycin isolated from *Streptomyces bambergiensis* whose isolation, characterization and biological properties have been reported[2~4], is a complex of several, chemically very similar components which include various sugars, a lipoid portion, an UV-chromophore and phosphorus bound in ester-like form. By means of column chromatography on silica gel, the moenomycin complex can be separated into components A, $B_1$, $B_2$ and C, using *n*-propanol - 2N ammonia as the eluant. Identification of the components separated in various fractions has been carried out by thin-layer chromatography on silica gel GF with isopropanol - 2N ammonia (70 : 30) as solvent system, spraying with chlorosulfonic acid - glacial acetic acid (1 : 2) and heating to 100°C, the moenomycins becoming visible as reddish-violet spots with different Rf-values[3].

Moenomycin A is the main component; when it is hydrolyzed with 2N hydrochloric acid, a crude lipoid fraction, two amino sugars, two neutral sugars and the UV-chromophore are split off. From the lipoid fraction three compounds could be isolated and identified[5,6]: moenocinol—a $C_{25}$-polyisoprenoid with five isolated double bounds—its allyl rearrangement product, and the olefin moenocene obtained from it by the splitting off of water. The amino sugars were identified as D-glucosamine and D-quinovosamine, the neutral sugar fraction contains D-glucose and a substance $Z_1$ whose structure is still unknown[7]. The UV-chromophore belongs to the group of amino reductones and possesses the structure of a 2-amino-cyclopentane-1,3-dione[1].

A comparison of the biological, chemical and physical properties of the moenomycins with the recently described prasinomycins[8~10] shows that related compounds

are involved; the lipoid substances obtained by hydrolysis of prasinomycin are identical with the moenocinol, isomoenocinol and moenocene isolated from moenomycin hydrolysate[10].

## Materials and Methods

Preparation of components E and F from moenomycin $B_1$, by means of preparative thin-layer chromatography:

A solution of 30 mg moenomycin $B_1$ in 2 ml methanol – water (1 : 1) is applied with a capillary pipette in the form of a narrow band to the starting line of 20×20 cm silica gel HF (E. Merck A. G., Darmstadt, Germany) thin-layer plates; the solution is distributed over 30 plates, *i. e.* 1 mg of the substance per plate. The chromatograms are developed in closed chambers in the chloroform – ethanol – water system (40 : 70 : 20). For the subsequent localization of the peak, a narrrow strip, parallel to the flow direction, in the middle as well as along the edge of the thin-layer plate, is sprayed with a solution of antimony chloride in chloroform. The zones made visible in the middle and on the edge indicated the peaks of moenomycin E and F to be marked on the thin-layer chromatogram.

The two substance-containing bands are lifted off from the glass plate; the sprayed bands are left out. By repeated swirling in methanol and centrifuging, the moenomycin E or F adsorbed on the silica gel is leached out and the methanolic solution evaporated *in vacuo* to dryness. The residue is taken up in a little methanol (about 5 ml), filtered, and the moenomycin precipitated with ether from the filtrate which is made slightly alkaline with ammoniacal methanol. By centrifuging the precipitate, washing with ether and drying, moenomycins E (11 mg) and F (8.5 mg) are obtained as ammonium salts.

Preparation of components E and F from moenomycin $B_1$ by means of ion-exchange chromatography:

Moenomycin is adsorbed on an anion-exchange column consisting of 100 ml Dowex 1 (chloride form; particle size 200~400 mesh) from a solution of 500 mg moenomycin $B_1$ in 5 ml water which has been adjusted to pH 8.0 with 2N ammonia. The column is washed with 100 ml water, subsequently with 100 ml of a mixture of methanol – water (4 : 1). The column is then eluted with 600 ml of a 0.6% potassium chloride solution in methanol – water (4 : 1), the volume per fraction is 15 ml; the drop rate is 30 ml per hour. The individual fractions are evaluated by thin-layer chromatography on silica gel GF in the chloroform – ethanol – water system (40 : 70 : 20) and by making the spots visible with chlorosulfonic acid – glacial acetic acid (1 : 2) as the spray reagent. Under these conditions, moenomycin E is found in fractions 8~14, moenomycin F in fractions 23~36; mixed fractions do not occur.

Moenomycins E and F are isolated in the following way from the two indicated fractions: first the methanol is removed by evaporation *in vacuo* and then the remaining aqueous solution is subjected to dialysis for 24 hours to separate the moenomycin from the potassium chloride; in this process the greater part of the moenomycin remains in the internal dialyzate owing to its property of associating in neutral, aqueous solution to particles of high molecular weight. The dialyzed moenomycin solution is then carefully evaporated to dryness in high vacuum, the residue taken up with 10 ml methanol, the solution filtered and the moenomycin precipitated from the filtrate by mixing with the threefold volume of ether. The precipitate is separated by centrifuging, washed with ether and dried. 105 mg moenomycin E and 136 mg moenomycin F in the form of the potassium salts are obtained.

Preparation of components G and H from moenomycin $B_2$ by ion-exchange chromatography:

Moenomycin $B_2$ (500 mg) is dissolved in water and after adjusting the pH to 8.0, adsorbed from this solution on 100 ml Dowex 1 (chloride form; 200~400 mesh). The column is

consecutively washed with 100 ml water and 100 ml methanol – water (4 : 1) and the moenomycin components G and H fractionally desorbed by elution with 600 ml of a 0.6% potassium chloride solution in methanol – water (4 : 1). Fractions, each of 15 ml, are collected and the drop rate so adjusted that 30 ml of eluate are obtained per hour. On the basis of the thin-layer chromatography test on silica gel GF in the chloroform – ethanol – water system (40 : 70 : 20), the components G and H are distributed over the following fractions: moenomycin G over fractions 9~17 and moenomycin H over fractions 24~43.

In analogy to the preceding example, moenomycin G or H is obtained from the combined fractions by evaporation of the methanol, removal of the potassium chloride by dialysis of the aqueous solution, freeze-drying of the internal dialyzate and precipitation of the residue from methanolic solution by the addition of ether. Centrifuging and drying of the precipitate yields 76 mg moenomycin G and 128 mg moenomycin H in the form of the potassium salts.

Preparation of moenomycin D by column chromatography on silica gel:

A crude fraction (200 mg) consisting of moenomycin $B_1$ and D is adsorbed from aqueous solution on 1 g silica gel (particle size 60~75 $\mu$) by evaporation of the water. The dry powder is placed on top of a column to which a suspension of 40 g silica gel (particle size 60~75 $\mu$) in isopropanol had previously been applied. Chromatographic development is carried out first with 150 ml isopropanol – 2N ammonia (10 : 2) and then with (9 : 2); finally elution is carried out with 750 ml of a mixture of isopropanol – 2N ammonia (80 : 25) with simultaneous fractionation. The volume per fraction is 10 ml, the drop rate 20 ml per hour. Thin-layer chromatographic analysis of the individual fractions on silica gel GF in the chloroform – ethanol – water (40 : 70 : 20) and isopropanol – water – borate buffer pH 9.0 (70 : 25 : 5) systems shows that initially mixed fractions consisting of moenomycins D, E and F or D and F occur: fractions Nos. 57~65 contain component D exclusively.

To isolate moenomycin D, the combined fractions 57~65 are evaporated to dryness *in vacuo*: the residue is dissolved in methanol and after filtering, the antibiotic is precipitated by addition of an excess of ether. The precipitate is centrifuged off, washed with ether and dried. In this way moenomycin D is obtained as the ammonium salt (34 mg).

## Results and Discussion

### Separation of the Moenomycins $B_1$ and $B_2$ into Components D, E, F, G and H

The moenomycin complex can be fractionated into the four components A, $B_1$, $B_2$ and C by silica gel chromatography with propanol-ammonia mixtures[3]. Moenomycins A and C are characterized by an UV-absorption at 258 m$\mu$ whilst this absorption band is lacking in the components $B_1$ and $B_2$.

The use of other solvent systems in thin-layer chromatography on silica gel GF results in further separation of moenomycin $B_1$ and $B_2$ into two components each: moenomycin $B_1$ is separated into components E and F, moenomycin $B_2$ into components G and H. An additional component D can be obtained from crude $B_1$ fractions which exhibit weak UV-absorption; moenomycin D, like components A and C, exhibits a pronounced UV-absorption maximum at 258 m$\mu$. The mixtures chloroform – ethanol – water (40 : 70 : 20) and isopropanol – water – borate buffer pH 9.0 (70 : 25 : 5) are suitable for these separations by thin-layer chromatography.

Table 1 gives a survey of the chromatographic behaviour of the newly discovered moenomycin components on silica gel thin-layer plates. With the aid of the solvent systems listed in the table, it is possible to separate and identify the afore-mentioned

moenomycins by means of single or repeated thin-layer chromatography which can also be performed two-dimensionally on one silica gel plate.

Isolation of the individual components in small quantities is also possible by means of preparative thin-layer chromatography on silica gel, using the chloroform - ethanol - water (40 : 70 : 20) system.

Table 1. Behaviour of moenomycins D, E, F, G and H in thin-layer chromatography on silica gel GF.

| Solvent system* | Rf-values of the moenomycins | | | | |
|---|---|---|---|---|---|
| | D | Moenomycin $B_1$ | | Moenomycin $B_2$ | |
| | | E | F | G | H |
| I | 0.36 | 0.36 | 0.36 | 0.30 | 0.30 |
| II | 0.20 | 0.25 | 0.14 | 0.22 | 0.14 |
| III | 0.48 | 0.67 | 0.47 | 0.65 | 0.35 |

* Solvent systems:
I = Isopropanol - 2N ammonia (70 : 30)
II = Chloroform - ethanol - water (40 : 70 : 20)
III = Isopropanol - water - borate buffer pH 9.0 (70 : 25 : 5)

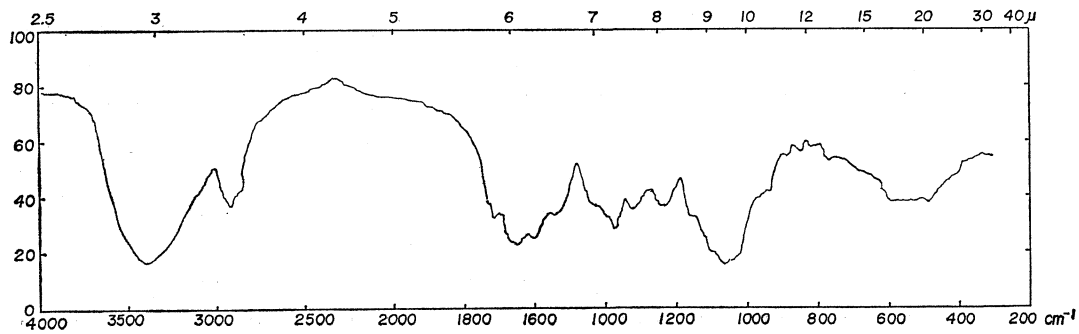
Component D which is present only in traces can also be prepared in the pure state by chromatography of a mixed fraction consisting of moenomycin $B_1$ (=E+F) and D, on silica gel columns with isopropanol - 2N ammonia as the eluant.

Column chromatography on anion-exchangers has so far proved to be the best method for the preparative isolation of components D, E, F, G and H. From an ammoniacal solution adjusted to pH 8.0, the moenomycin $B_1$ or $B_2$ is first adsorbed on the exchanger resin (Dowex-1) in chloride form. After washing with water and aqueous methanol, the individual components are fractionally eluted with a solution of 0.6 % potassium chloride in methanol - water (4 : 1). After distillation of methanol, followed by dialysis from potassium chloride and elimination of the water by distillation under high vacuum, the moenomycins are obtained as potassium salts by addition of ether to their methanolic solutions.

## Physical and Chemical Properties of the Moenomycins

Moenomycins D, E, F, G and H are colourless, amorphous and weakly acidic substances with very similar properties. They are particularly characterized by their phosphorus content of about 1.9 %. Moenomycin D, like moenomycins A and C, exhibits a pronounced absorption maximum at 258 m$\mu$ ($E^{1\%}_{1cm}$ 49) in phosphate buffer pH 7.0 and thus differs from components E, F, G and H which do not have a characteristic UV-absorption above 220 m$\mu$. The IR-spectra only show slight differences in the location and intensity of their bands; Fig. 1 gives as an example the IR-spectrum of moenomycin F in potassium bromide.

Fig. 1. Infrared spectrum of moenomycin F in potassium bromide.



All moenomycins are readily soluble in water, in lower aliphatic alcohols and in other polar organic solvents such as dimethyl formamide and glacial acetic acid. They form water-soluble salts with alkali metals.

The moenomycins are stable in neutral, aqueous and methanolic solutions but decompose slowly in an acid or alkaline medium. By acid hydrolysis, first the lipoid portion is split off which can be extracted with chloroform. In the case of drastic acid hydrolysis, besides a mixture of phosphoric acid esters, it is possible to identify several neutral sugars and amino sugars, *e.g.* D-glucose, D-glucosamine and D-quinovosamine; in the hydrolysate of moenomycin G there is no D-quinovosamine and in the hydrolysate of components F and H glycine is detectable in addition to the sugars named.

Thin-layer chromatography on silica gel GF is suitable for the rapid identification of the moenomycin components; the Rf-values in three different solvent systems are compiled in Table 1. In addition the moenomycins are characterized by the following data:

Moenomycin D: Elementary analysis ($NH_4$-salt): C 43.7, H 7.1, N 5.7, P 1.9. IR-spectrum (in potassium bromide): 2.95, 3.42, 5.82, 6.02, 6.45 (shoulder), 7.13, 7.55, 9.40 $\mu$.

Moenomycin E: Elementary analysis (K-salt): C 46.3, H 6.7, N 4.4, P 1.8, K 2.6. IR-spectrum (in potassium bromide): 2.95, 3.38 (shoulder), 3.42, 5.85, 6.00, 7.30, 7.58, 8.10, 9.40 $\mu$.

Moenomycin F: Elementary analysis (K-salt): C 44.9, H 5.4, N 4.0, P 1.7, K 4.5. IR-spectrum (in potassium bromide): 2.95, 3.38 (shoulder), 3.42, 5.85, 6.02, 6.10, 6.48, 7.28, 7.55, 8.12, 9.40 $\mu$.

Moenomycin G: Elementary analysis (K-salt): C 47.2, H 6.4, N 4.6, P 2.0, K 2.3. IR-spectrum (in potassium bromide): 2.95, 3.42, 5.82, 6.00, 6.43 (shoulder), 7.28, 7.58, 8.15, 9.40 $\mu$.

Moenomycin H: Elementary analysis (K-salt): C 46.1, H 6.3, N 4.1, P 1.9, K 4.3. IR-spectrum (in potassium bromide): 2.95, 3.42, 5.83, 6.02, 6.13 (shoulder), 7.25, 7.58, 8.20, 9.40 $\mu$.

## Biological Properties of the Moenomycins

The spectrum of action of moenomycins E, F, G and H compared with the moenomycin complex as determined by a serial dilution test is shown in Table 2. The individual components exhibit an increased antibiotic effect against some test

Table 2. Antimicrobial activity of the moenomycins.

| Test organism | Minimum inhibitory concentration of moenomycin components ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|
| | E | F | G | H | Complex |
| *Staphylococcus aureus* FDA 209P | 0.008 | 0.06 | 0.06 | 0.06 | 0.05 |
| *Staph. aureus*, penicillin-resistant | 0.005 | 0.03 | 0.03 | 0.03 | 0.05 |
| *Bacillus subtilis* | 0.0025 | <0.0025 | 0.0025 | <0.0025 | 0.1 |
| *Streptococcus faecalis* 1140 | 3.0 | 0.1 | 0.025 | 3.0 | 2.0 |
| *Escherichia coli* | 8 | 15 | 8 | 31.5 | 188 |
| *Proteus vulgaris* | >250 | 125 | 15 | 60 | 24 |
| *Mycobacterium* 607 | 6 | 2 | 2 | 2 | 4 |
| *Candida albicans* | >250 | >250 | >250 | >250 | >250 |

bacteria, especially against *Escherichia coli* and *Bacillus subtilis*. The antibiotic activity of moenomycin D corresponds to that of the moenomycin complex[4].

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