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On the biosynthesis of moenocinol, the lipid part of the moenomycin antibiotics

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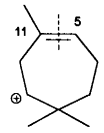
Abstract

The lipid part of the moenomycins is completely isoprenoid and is constructed via the non-mevalonate pathway. The central C_{10} part originates from a precursor like geranyl or linalyl diphosphate and is formed by a route involving ring formation between C-2 and C-6 of the monoterpene unit, two successive rearrangements to give a seven-membered ring intermediate and cleavage of the ring at the C-5 and C-11 bond (moenocinol numbering).

The moenomycin-type antibiotics[1] contain a C_{25} lipid unit (the moenocinol part, see formula **2**, R=remainder of the moenomycin molecule) that has an interesting structure. Three isoprenoid C_5 units are easily discernible, whereas the central C_{10} part (C-5 through C-11) does not obey the isoprene rule in an obvious way. It has been speculated a long time ago[2] that this C_{10} unit could be formed by *anti*-Markovnikov cyclization of a geranyl-type diphosphate to give a structure of type **A** and opening of the bond between C-5 and C-11 (moenocinol numbering).



A

Recently, we performed preliminary [1-^{13}C]acetate feeding experiments with cultures of *Streptomyces ghanaensis* H2 (semi-producing strain from the BC Biochemie GmbH collection[3]). The usual mixture of moenomycins[1] (containing moenomycin A as the major component) was isolated and, although the ^{13}C enrichments were very low, the labeling pattern shown in Scheme 1 could be identified.[4] The following conclusions were drawn from this experiment:

(i) The moenocinol unit is formed via the non-mevalonate pathway.[5]

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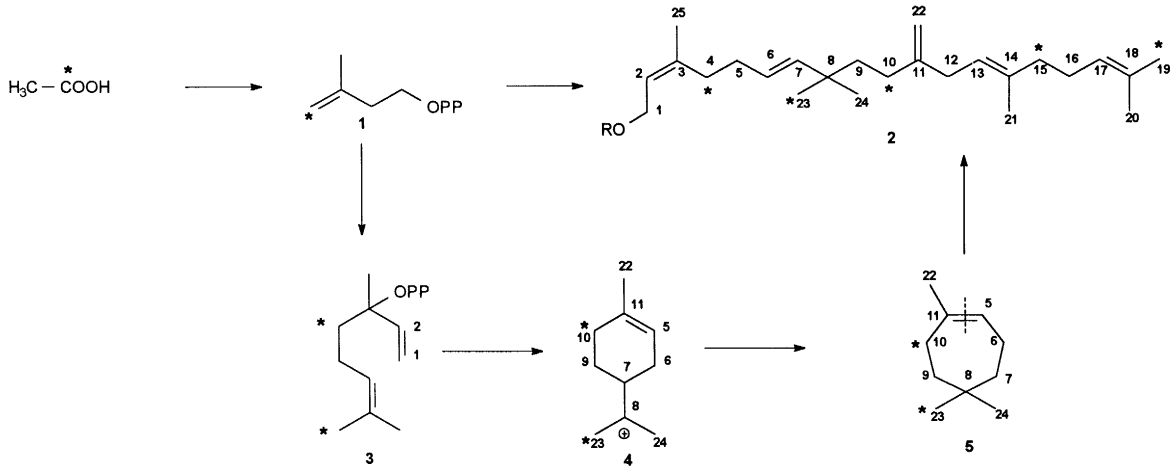


Scheme 1.

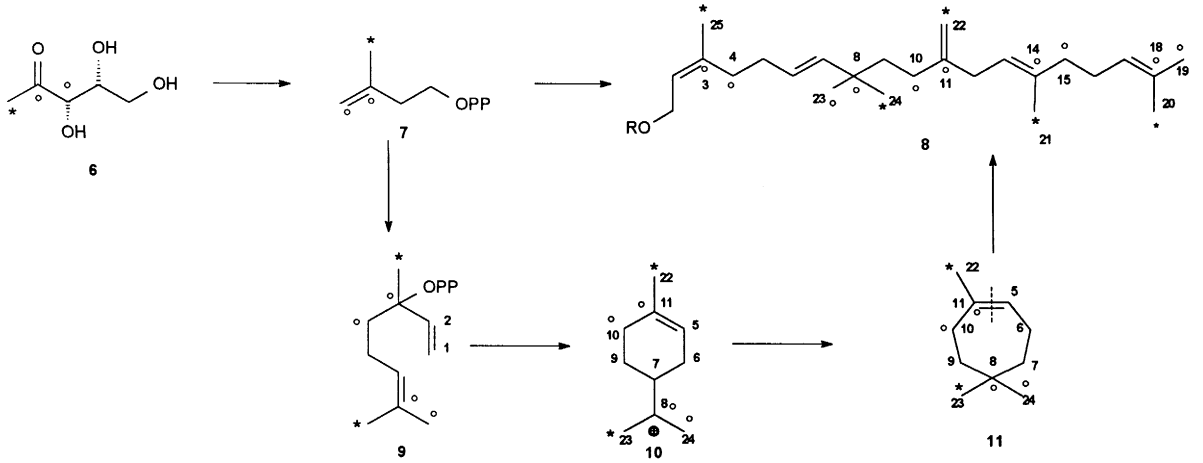
(ii) The central part results from cyclization of linalyl diphosphate in the Markovnikov sense, followed by migration of the bond between C-7 and C-9 (moenomycin numbering) to give a seven-membered intermediate of type **5** and cleavage of the bond between C-5 and C-11 of **5** (see Scheme 1).

In the present Communication we wish to describe feeding experiments that allow a much more evolved view on the biosynthesis of the moenomycin lipid part.

In a first set of experiments cultures of *Streptomyces ghanaensis* H2 were grown in Erlenmeyer flasks (medium 1,[6] 750 mL). After 48 h a single dose of [1-^{13}C]-1-deoxy-D-xylulose (**6** with starred position labeled, 150 mg) was administered. The fermentation was stopped after 240 h and the mixture of the moenomycins was isolated.[7] ^{13}C NMR spectra were recorded in 10:1 methanol:water. Well-resolved spectra were obtained. All signals of the lipid part could be assigned by comparison with previous results.[8] For a quantitative analysis the inverse gated decoupling ^{13}C NMR spectrum of the unlabelled moenomycin mixture was recorded under the same conditions. The enrichments were calculated comparing the corresponding signals (referenced to C-2 of moenomycin unit A[4]) of labeled and unlabeled moenomycins using a known procedure.[9] The following positions (starred positions in **8**, Scheme 2) were enriched: C-20 (2.0%), C-21 (1.3%),[10] C-23/C-24[11] (3.6%), C-22 (3.1%), C-25 (3.1%). This result proves the previous assumption that all units of the moenocinol part are isoprenoid and formed via the non-mevalonate pathway.

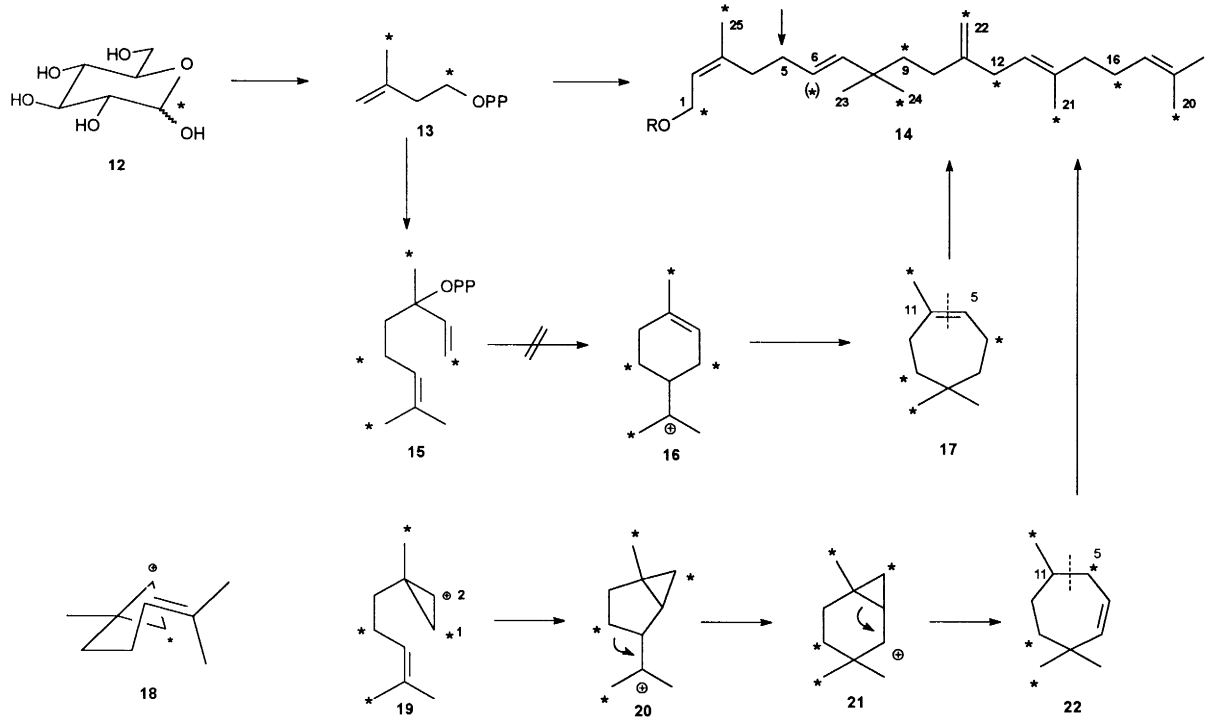
In the second feeding experiment under identical conditions [2,3-$^{13}C_2$]-1-deoxy-D-xylulose[12] (**6**, with circled positions labeled) was administered (50 mg). The ^{13}C NMR spectrum of the isolated moenomycin mixture displayed the labeling pattern shown in Scheme 2 (circled positions: C-19 (1.1%, $^1J_{19,18}$=43.3 Hz), C-23/C-24 (not separated, 0.6%, $^1J_{23/24,8}$=35.4 Hz), C-10 (1.7%, $^1J_{10,11}$=41.5 Hz), C-4 (1.1%, $^1J_{4,3}$=41.5 Hz), C-8 (0.9%, $^1J_{8,23/24}$=35.4 Hz), C-15 (1.3%, $^1J_{15,14}$=42.4 Hz), C-18 (0.4%, $^1J_{18,19}$=43.3 Hz), C-14 (0.8%, $^1J_{14,15}$=42.4 Hz), C-3 (0.7%, broad non-resolved signal), C-11 (1.0%, $^1J_{11,10}$=41.5 Hz)). The result is clearly in agreement with the conclusions taken from the feeding experiments discussed above.

In a third feeding experiment [1-^{13}C]-D-glucose was administered using a somewhat different protocol.[13] From the ^{13}C NMR spectra the labeling pattern summarized in Scheme 3 was obtained: C-21 (0.6%), C-20 (0.4%), C-25 (0.6%), C-16 (0.5%), C-23/C-24 (0.2%), C-5 (0.5%), C-12 (0.3%), C-9 (0.4%), C-22 (0.2%).[14] With one exception the labels were found in the expected positions. However, contrary to expectations, C-5 was labeled rather than C-6. This result means that in the course of the



Scheme 2.

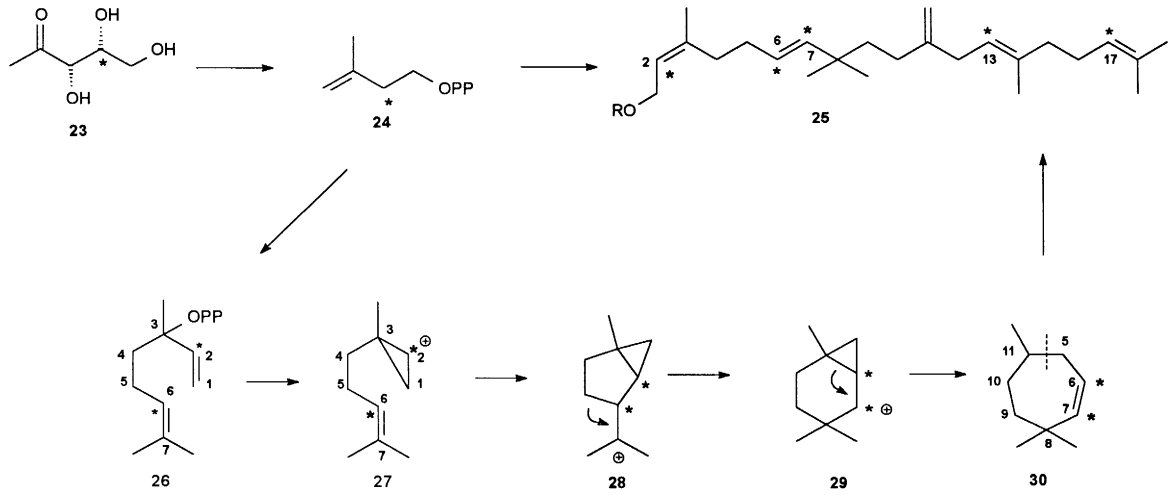
formation of the seven-membered intermediate from linalyl or geranyl diphosphate, carbons 1 and 2 (geraniol numbering) must exchange their positions. A three-membered ring intermediate (see **18** and **19** in Scheme 3) appears to offer a reasonable explanation.



Scheme 3.

With the aim of substantiating the mechanistic rationale summarized in Scheme 3, [4-^{13}C]-1-deoxy-D-xylulose[12] (**23**, 160 mg) was administered under the conditions of experiments 2 and 3 (vide supra). Using the usual procedure in the moenocinol part the labeling pattern summarized in Scheme 4 was found to be: C-2 (1.7%), C-13 (2.3%), C-17 (1.8%), C-6 (1.3%, $^{1}J_{6,7}$=72.2 Hz), C-7 (1.2%, $^{1}J_{7,6}$=72.2 Hz). This result nicely proves that carbons 2 and 6 (geraniol numbering) of the geraniol/linalool-derived

intermediate **27** are joined in the course of the formation of moenocinol and become moenocinol carbons 6 and 7 (see **30** and **25**), respectively.



Scheme 4.

In conclusion, we have shown that the lipid part of the moenomycins is completely isoprenoid and is constructed via the non-mevalonate pathway. The formation of the central C_{10} part originates from a precursor like geranyl or linalyl diphosphate and proceeds via a pathway as shown in Scheme 3 involving: (i) a ring formation between C-2 and C-6 (geraniol numbering); (ii) two successive rearrangements to give a seven-membered ring intermediate; and (iii) cleavage of the bond between C-5 and C-11 (moenocinol numbering).

Acknowledgements

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1. For leading references, see: Donnerstag, A.; Marzian, S.; Müller, D.; Welzel, P.; Böttger, D.; Stärk, A.; Fehlhaber, H.-W.; Markus, A.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1995**, *51*, 1931–1940.
2. Böttger, D.; Welzel, P. *Tetrahedron Lett.* **1983**, *24*, 5201–5204.
3. BC Biochemie GmbH, Industriepark Höchst, 65929 Frankfurt/M.
4. Endler, K.; Schuricht, U.; Hennig, L.; Welzel, P.; Holst, U.; Aretz, W.; Böttger, D.; Huber, G. *Tetrahedron Lett.* **1998**, *39*, 13–16.
5. For a review, see: Rohmer, M. *Nat. Prod. Rep*. **1999**, *16*, 565–574.
6. Medium 1: cornsteep liquor (3.3 g), soybean meal (6.4 g), $CaCO_3$ (1.65 g), soybean oil (11.7 g), $(NH_4)_2SO_4$ (1.5 g), glucose (7.5 g), amylase bac. (0.5 mg), $CoSO_4$ (0.7 mg), KH_2PO_4 (50 mg), Genapol® (20% in water, 12.5 g), deion. water (0.75 L), pH 7. Preculture medium: cornsteep liquor (0.4 g), soybean meal (3.0 g), $CaCO_3$ (0.45 g), soybean oil (0.4 g), glucose (5.2 g), KH_2PO_4 (0.1 g), deion. water (0.1 L), pH 7. Additional glucose was administered after 12 h (15 g/L), 24 h (7.5 g/L) and 30 h (7.5 g/L). All fermentations were performed at 37°C, shaker.

7. Cells were separated from the medium by filtration. The filtered solution was concentrated at 40°C (rotatory evaporator) and the residue was stirred with an ice-cold 8:2 methanol:water mixture for 2 h and then filtered. Cell disintegration was achieved by sonication in ice-cold 8:2 methanol:water. After filtration the filtrates were combined and methanol was evaporated. After setting the pH to 7.5 the aqueous solution was extracted with three portions of 1-butanol. Solvent evaporation from the aqueous phase, taking up the residue in 4:6 acetonitrile-buffer ($K_2HPO_4 \cdot 3H_2O$ (13.1 g), KH_2PO_4 (0.3 g) and water, final volume: 1 L), adjusting the pH to 7.5, and medium pressure LC (RP18, solvent: acetonitrile–buffer as described above) gave a fraction that was desalted by solid phase extraction (RP18, first water, then 1:1 acetonitrile:water). Acetonitrile removal by distillation and subsequent lyophilization provided the pure mixture of the moenomycins.
8. See compound M_A in: Kempin, U.; Hennig, L.; Welzel, P.; Marzian, S.; Müller, D.; Fehlhaber, H.-W.; Markus, A.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1995**, *51*, 8471–8482. The chemical shifts of C-19^I and C-20^I have to be reversed.
9. Scott, A. I.; Townsend, C. A.; Okada, K.; Kajiwara, M.; Crushley, R. J.; Whitman, P. J. *J. Am. Chem. Soc*. **1974,** *96*, 8069–8080.
10. The C-21 signal was not separated from the signal of the C-3 methyl group of unit F. Therefore, an exact integration was impossible.
11. C-23 and C-24 have the same chemical shift.
12. The synthesis of **6** and **23** will be reported in the full paper.
13. Gyratory shaker, medium 1.[6] For the feeding experiments with 1-^{13}C-glucose medium 1 was used. Additional glucose was administered after 12 h (15 g/L), 24 h (7.5 g/L including 2 g of [1-^{13}C]-D-glucose) and 30 h (7.5 g/L including 2 g of [1-^{13}C]-D-glucose).
14. The signal of C-1 was hidden by signals of the sugar part.