A Novel Type of Non-enzymatic Reaction during the Late Steps in the Biosynthesis of the Angucycline Antibiotics Urdamycins C and D

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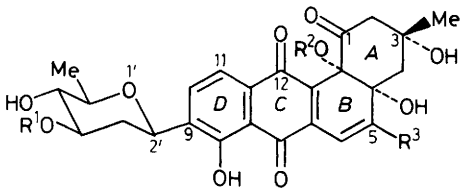
Investigations on the late steps in the biosynthesis of the urdamycin complex showed the surprising result that the interconversion of urdamycin A (**1**) into the urdamycins C (**2**) and D (**3**) are due to non-enzymatic condensations of (**1**) with 4-hydroxyphenylpyruvic acid and indole-3-pyruvic acid, respectively.

Angucyclines[1] containing aquayamycin[2,3] (**4**) as the aglycone moiety are the biologically most potent subgroup among this increasing family of antibiotics. Recently, representatives of this group were discovered during a screening for inhibitors of platelet aggregation[4,5] or for antitumour antibiotics.[6] The main compound of the urdamycin complex, produced by *Streptomyces fradiae* (Tü 2717), is the orange coloured urdamycin A (**1**) in which aquayamycin (**4**) is included as aglycone moiety.[7] Biosynthetic studies[8] showed urdamycin A (**1**) to be the precursor of the darker coloured urdamycins C [(**2**), dark red], D [(**3**), blue], and E [(**5**) red], and that these interconversions involve the amino acids tyrosine, tryptophan, and methionine, respectively. In the last case it could be shown that a non-enzymatic process, namely the nucleophilic Michael addition of methylmercaptan (liberated enzymatically from methionine) at the 5-position of the urdamycin A (= aquayamycin) chromophore, is the key reaction.[9]

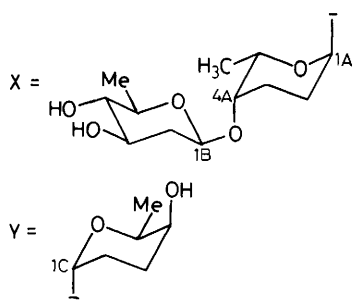
In this paper we report that another non-enzymatic reaction may be involved in the formation of the urdamycin C (**2**) and D (**3**) chromophores[10] from urdamycin A (**1**). For these interconversions several mechanisms (most of them proposing the involvement of an enzyme, like an oxygenase or an esterase) were considered but rejected as a result of different experiments.

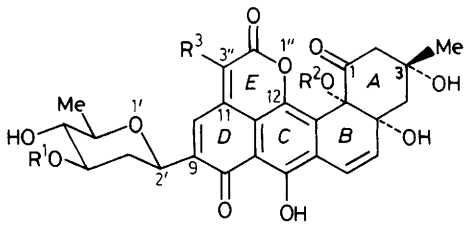
Finally, a mechanism involving urdamycin A (**1**) and oxidized pyruvic acid derivatives, (namely 4-hydroxyphenylpyruvic acid and indole-3-pyruvic acid easily formed *in vivo* by transamination from tyrosine and tryptophan, respectively) was proposed. Electrophilic attack of the oxidized pyruvic acid derivatives at the 11-position of urdamycin A (**1**), followed by closure of a δ-lactone ring (ring *E*) would result in the formation of urdamycin C (**2**) or D (**3**), as exemplified in Scheme 1, for (**2**).

Evidence for this mechanism was provided by the following

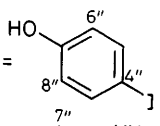


(**1**) R^1 = X, R^2 = Y, R^3 = H
(**4**) R^1 = H, R^2 = H, R^3 = H
(**5**) R^1 = X, R^2 = Y, R^3 = SMe

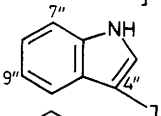




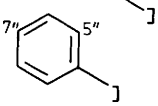
(**2**) R^1 = X, R^2 = Y, R^3 =

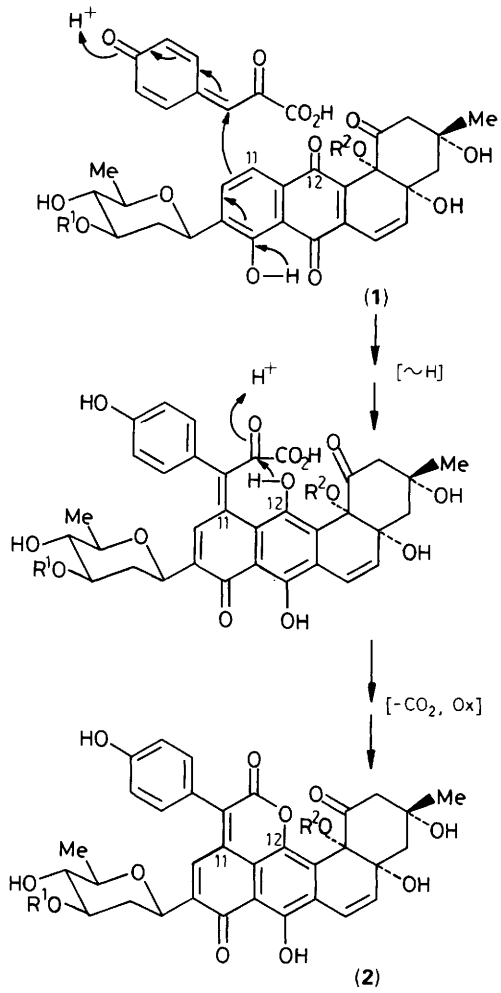


(**3**) R^1 = X, R^2 = Y, R^3 =



(**6**) R^1 = X, R^2 = Y, R^3 =





Scheme 1

in vitro experiment. Urdamycin A (**1**) was dissolved in dimethylsulphoxide (DMSO)–phosphate buffer (0.1 M) [1 : 10, or tetrahydrofuran (THF) instead of DMSO] and treated with a ten molar excess of 4-hydroxyphenylpyruvic acid at 28 °C (stirring in an open Erlenmeyer flask in order to allow contact with molecular oxygen). Urdamycin C (**2**) was formed quantitatively within 3 h. Using indole-3-pyruvic acid instead of 4-hydroxyphenylpyruvic acid gave urdamycin D (**3**) quantitatively within 1 h. The urdamycins C (**2**) and D (**3**) prepared in this way are totally identical with those obtained from *Streptomyces fradiae* (Tü 2717).

The mechanism was further supported, firstly, by experiments with the less readily oxidizable phenylpyruvic acid, which gave only 5% of 7″-deoxyurdamycin C (**6**) within 24 h under the same conditions as above; and secondly, by the fact that there is no reaction in a nitrogen atmosphere after titration of *e.g.*, indole-3-pyruvic acid with sodium dithionite. Complete reduction of the indole-3-pyruvic acid which is usually, at least partially, oxidized (orange colour) due to molecular oxygen is indicated by the change in colour of the solution from orange to yellow. Further experiments verifying the proposed mechanism are the subject of present investigations, along with possible applications of this reaction using modified acceptor molecules (urdamycin A derivatives, other than those discussed here), or other (oxidizable) α-keto acids.

After the formation of urdamycin E (**5**) from (**1**)[9] this is the second non-enzymatic reaction of urdamycin A (**1**) with 'enzymatically converted' amino acids (here simply transamination). As already suggested in the context of the formation of (**5**) [in which the aquayamycin-chromophore of (**1**) acts as an acceptor for a nucleophile], the reaction of urdamycin A (**1**) described here (acting as potent acceptor for electrophiles) might also be responsible for the mechanism of action of aquayamycin-containing antibiotics, and could thus explain their wide spectrum of interesting biological activities; *i.e.*, antitumour, antibacterial, enzyme inhibitory, platelet aggregation inhibition *etc.* Indeed urdamycin A (**1**) is biologically by far the most active compound among the urdamycins, whereas the urdamycins C (**2**) and D (**3**) show only weak activity.[1]

This novel type of reaction may be limited to systems close to the urdamycin A (= aquayamycin) chromophore as no analogues of urdamycins C (**2**) or D (**3**) derived from urdamycin E (**5**) or B† are found in the culture broth of *Streptomyces*.[1] In fact, treatment of urdamycin B with, *e.g.*, 4-hydoxyphenylpyruvic acid under the conditions described yielded no products.

The involvement of non-enzymatic reactions may also be considered for certain steps in the biosyntheses of other antibiotics (especially if there is a wide spectrum of related compounds), *e.g.*, the formation of the sulphur-containing side chain in some ansamycins[11—14] (non-enzymatic addition of methylmercaptan or gluthathione with subsequent degradation, evident after the work of Tanaka *et al.*[15,16]), or for some interconversion steps during the late biosynthesis of the naphthocyclinones,[17] and also for the formation of the racemic esmeraldin B (preferably from saphenamycin and saphenic acid).[18,19]

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References

1 H. Drautz, H. Zähner, J. Rohr, and A. Zeeck, *J. Antibiot.*, 1986, **39**, 1657.
2 M. Sezaki, S. Kondo, K. Maeda, H. Umezawa, and M. Ohno, *Tetrahedron*, 1970, **26**, 5171.
3 N. Imamura, K. Kakinuma, N. Ikekawa, H. Tanaka, and S. Omura, *Chem. Pharm. Bull.*, 1981, **29**, 1788.
4 S. Omura, A. Nakagawa, N. Fukamachi, S. Miura, Y. Takahashi, K. Komiyama, and B. Kobayashi, *J. Antibiot.*, 1988, **41**, 812.
5 A. Kawashima, Y. Yoshimura, J. Goto, S. Nakaike, T. Mizutani, K. Hanada, and S. Omura, *J. Antibiot.*, 1988, **41**, 1913.
6 Y. Hayakawa, T. Iwakiri, K. Imamura, H. Seto, and N. Otake, *J. Antibiot.*, 1987, **40**, 1785.
7 J. Rohr and A. Zeeck, *J. Antibiot.*, 1987, **40**, 459.
8 J. Rohr, J. M. Beale, and H. G. Floss, *J. Antibiot.*, 1989, **42**, 1151.
9 J. Rohr, *J. Chem. Soc., Chem. Commun.*, 1989, 492.
10 J. Rohr, A. Zeeck, and H. G. Floss, *J. Antibiot.*, 1988, **41**, 126.
11 M. Meyer, W. Keller-Schierlein, S. Megahed, H. Zähner, and A. Segre, *Helv. Chim. Acta*, 1986, **69**, 1356.
12 S. Funayama, K. Okada, H. Oka, S. Tomosaka, T. Miyano, K. Komiyama, and I. Umezawa, *J. Antibiot.*, 1985, **38**, 1285.
13 J. Mochizuki, E. Kobayashi, K. Furihata, A. Kawaguchi, H. Seto, and N. Otake, *J. Antibiot.*, 1986, **39**, 157.
14 M. Hotta, Y. Hayakawa, K. Furihata, A. Shimazu, H. Seto, and N. Otake, *J. Antibiot.*, 1986, **39**, 311.
15 T. Okabe, B. D. Yuan, F. Isono, I. Sato, H. Fukazawa, T. Nishimura, and N. Tanaka, *J. Antibiot.*, 1985, **38**, 230.
16 T. Okabe, K. Suzuki, H. Suzuki, Y. Inouye, S. Nakamura, and N. Tanaka, *J. Antibiot.*, 1986, **39**, 316.
17 B. Krone, A. Zeeck, and H. G. Floss, *J. Org. Chem.*, 1982, **47**, 4721.
18 W. Keller-Schierlein, A. Geiger, H. Zähner, and M. Brandl, *Helv. Chim. Acta*, 1988, **71**, 2058.
19 A. Geiger, W. Keller-Schierlein, M. Brandl, and H. Zähner, *J. Antibiot.*, 1988, **41**, 1542.

† Structure as (**1**) but with ring *B* aromatic.