# Biosynthesis of the dialkylmaleic anhydride-containing antibiotics, tautomycin and tautomycetin

**Makoto Ubukata,*[a,b] Xing-Chun Cheng,[b] Jun Uzawa[b] and Kiyoshi Isono[c]**

[a] *Biotechnology Research Center, Toyama Prefectural University, Kosugi-machi, Imizu-gun, Toyama 939-03, Japan*
[b] *The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama 351-01, Japan*
[c] *Department of Marine Science, School of Marine Science and Technology, Tokai University, 3-20-1, Orido, Shimizu, Shizuoka 424, Japan*

The biosynthetic origins of tautomycin and tautomycetin produced by *Streptomyces spiroverticillatus* and *Streptomyces griseochromogenus*, respectively, have been studied by feeding experiments with $^{13}C$ labelled precursors. The left half of tautomycin and tautomycetin are synthesized from one propionate and a C-5 unit. The latter is formed from three acetate units with decarboxylation. The labelling pattern from $^{13}C$-acetates indicated that α-keto glutarate or an equivalent may be a precursor. The results of a feeding experiment of [1,2-$^{13}C_2$]glutamate afforded the direct proof for this idea. The right half of tautomycin is biosynthesized by a polyketide pathway which starts with isobutyrate followed by introduction of a glycolate, five acetate and five propionate units. The terminal methyl carbon originates from an acetate-methyl probably formed by decarboxylation of the intermediate terminal β-keto carboxylate anion. The right half of tautomycetin is formed *via* a polyketide pathway which starts with acetate followed by introduction of three acetate and four propionate units and one butyrate unit.

The antifungal antibiotics, tautomycin[1] and tautomycetin[2] were found to be produced by *Streptomyces spiroverticillatus* and *Streptomyces griseochromogenus*, respectively. Both antibiotics have similar structures and biological activities. These two antibiotics have not only a unique 2-(1-hydroxy-2-carboxyethyl)-3-methylmaleic anhydride structure,[3,4] but also have unique biological activity. On further investigation, it was found that tautomycin and tautomycetin induced blebs on the cell surface of human leukemia cell K562.[5] More recently it was found that tautomycin inhibits protein phosphatase activity in a cell-free system,[6-8] and competes with the specific binding of [$^3$H]labelled okadaic acid to a particulate fraction prepared from mouse skin.[6] Because of the increasing importance of tautomycin as a strong specific inhibitor of protein phosphatases 1 and 2A, its stereochemistry was elucidated,[9] and a total synthesis of tautomycin has been recently completed.[10]

This paper deals with the biosynthesis of these unique polyketide antibiotics, tautomycin and tautomycetin (Fig. 1).

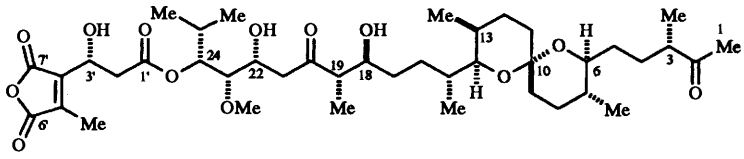
## Results and discussion

### Biosynthesis of tautomycin

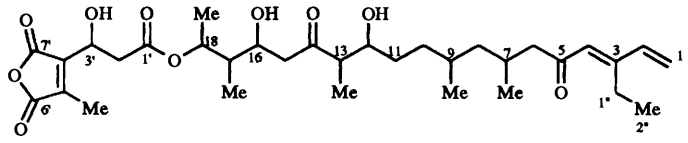
In the feeding experiments for a tautomycin-producing strain, *Streptomyces spiroverticillatus* sp. JC-84-44, sodium [1-$^{13}C$]-acetate, sodium [2-$^{13}C$]acetate, sodium [1,2-$^{13}C_2$]acetate, sodium [1-$^{13}C$]propionate, sodium [2-$^{13}C$]propionate, sodium [3-$^{13}C$]propionate, L-[methyl-$^{13}C$]methionine, sodium [1-$^{13}C$]isobutyrate, [1,2-$^{13}C_2$]glycine and sodium [1,2-$^{13}C_2$]glutamate were used as $^{13}C$-labelled precursors for tautomycin. The detailed $^{13}C$ enrichment data of tautomycin are listed in Table 1. Feeding experiments of sodium [1-$^{13}C$]acetate showed that C-4, C-8, C-10, C-16, C-20, C-1′ and C-7′ were derived from sodium [1-$^{13}C$]acetate. $^{13}C$ Enrichments were observed at C-1, C-2, C-3, C-5, C-6, C-7, C-9, C-11, C-12, C-13, C-14, C-15, C-17, C-18, C-19, C-21, C-2′, C-3′, C-4′, C-5′, C-6′, 3-Me, 7-Me, 13-Me, 15-Me, 19-Me, 25-Me and 5′-Me as a result of the feeding experiment with sodium [2-$^{13}C$]acetate. Relatively high enrichment ratios at C-1, C-5, C-9, C-11, C-17, C-21, C-2′ and C-4′ among them were observed as shown in Table 1. These results are consistent with the results of the feeding experiment of sodium [1-$^{13}C$]acetate and indicated that seven acetate units were incorporated into tautomycin. The lower enrichment values at C-2, C-3, C-6, C-7, C-12, C-13, C-14, C-15, C-18, C-19, C-5′, C-6′, 3-Me, 7-Me, 13-Me, 15-Me, 19-Me and 5′-Me indicated randomization of sodium [2-$^{13}C$]acetate to [1,2,3-$^{13}C_3$]propionate presumably by multiple passages through the Krebs cycle followed by conversion of succinate into propionate by methylmalonyl Co-A mutase as already observed in cationomycin[11] and cytovaricin biosynthesis.[12]

In the feeding experiments using $^{13}C$ propionate, C-2, C-6, C-12, C-14, C-18 and C-6′ were derived from sodium [1-$^{13}C$]propionate and 3-Me, 7-Me, 13-Me, 15-Me, 19-Me and 5′-Me were derived from sodium [3-$^{13}C$]propionate as shown in Table 1. These results indicated that six propionate units were incorporated into tautomycin. Additional evidence for the incorporation of $^{13}C$ propionate was obtained in the feeding experiment using sodium [2-$^{13}C$]propionate, which showed $^{13}C$ enrichment at C-3, C-7, C-13, C-15, C-19 and C-5′. The feeding experiment using sodium [1-$^{13}C$]isobutyrate showed that only C-24 was derived from C-1 of isobutyrate. In the feeding experiment using L-[methyl-$^{13}C$]methionine, high enrichment was observed at the methoxy methyl at C-23.

In the 2-D INADEQUATE[13] spectrum of tautomycin labelled with sodium [1,2-$^{13}C_2$]acetate, the pattern of carbon-carbon correlation observed by cross peaks between the coupled carbons due to the randomization of sodium [1,2-$^{13}C_2$]acetate revealed most of carbon connectivities of tautomycin. Although no enrichment could be detected on C-22 and C-23 in the 1D $^{13}C$ NMR of tautomycin labelled with sodium [1-$^{13}C$]acetate, sodium [2-$^{13}C$]acetate or sodium [1,2-$^{13}C_2$]acetate, the rather weak cross peaks of C-22 (66.4 ppm) and C-23 (80.6 ppm) were observed in the 2-D INADEQUATE spectrum of tautomycin labelled with sodium [1,2-$^{13}C_2$]acetate.[3] The low incorporation of sodium [1,2-$^{13}C_2$]acetate may indicate that acetate was metabolized to isocitrate which was converted into glyoxylate, followed by conversion into glycolate, namely hydroxyacetyl-Co A, then introduced into C-22 and C-23. In the leucomycin biosynthetic



Tautomycin



Tautomycetin

**Fig. 1** Structures of tautomycin and tautomycetin

**Table 1** $^{13}C$ Chemical shifts and isotopic incorporation into tautomycin

| Carbon No. | $\delta(^{13}C)$ | Relative enrichment[a] [1-$^{13}$C]-Acetate | [2-$^{13}$C]-Acetate | L-[Me-$^{13}$C]-Methionine | [1-$^{13}$C]-Propionate | [2-$^{13}$C]-Propionate | [3-$^{13}$C]-Propionate |
|---|---|---|---|---|---|---|---|
| 1 | 28.0 | — | 3.5 | — | — | | |
| 2 | 213.0 | — | 1.9 | — | 6.0 | — | — |
| 3 | 47.4 | — | 1.6 | — | — | — | — |
| 4 | 29.1 | 3.6 | — | — | — | 2.0 | — |
| 5 | 30.7 | — | 3.7 | — | — | — | — |
| 6 | 74.3 | — | 1.8 | — | 4.0 | — | — |
| 7 | 34.9 | — | 2.0 | — | — | — | — |
| 8 | 28.1 | 3.0 | — | — | — | 1.7 | — |
| 9 | 36.1 | — | 3.9 | — | — | — | — |
| 10 | 95.4 | 3.5 | — | — | — | — | — |
| 11 | 30.2 | — | 3.9 | — | — | — | — |
| 12 | 26.7 | — | 2.4 | — | 3.8 | — | — |
| 13 | 27.6 | — | 2.7 | — | — | 2.0 | — |
| 14 | 74.8 | — | 1.9 | — | 5.2 | — | — |
| 15 | 34.8 | — | 2.6 | — | — | 1.7 | — |
| 16 | 27.4 | 3.3 | — | — | — | — | — |
| 17 | 31.4 | — | 3.3 | — | — | — | — |
| 18 | 74.3 | — | 1.8 | — | 4.0 | — | — |
| 19 | 52.4 | — | 1.6 | — | — | 2.0 | — |
| 20 | 215.0 | 4.4 | — | — | — | — | — |
| 21 | 45.8 | — | 4.4 | — | — | — | — |
| 22 | 66.4 | — | — | — | — | — | — |
| 23 | 80.6 | — | — | — | — | — | — |
| 24 | 76.5[b] | — | — | — | — | — | — |
| 25 | 28.7 | — | — | — | — | — | — |
| 26 | 19.4 | — | 1.53 | — | — | — | 4.0 |
| 1′ | 196.6 | 5.7 | — | — | — | — | — |
| 2′ | 40.9 | — | 6.3 | — | — | — | — |
| 3′ | 64.0 | — | 2.7 | — | — | — | — |
| 4′ | 142.1 | — | 3.2 | — | — | — | — |
| 5′ | 142.9 | — | 1.8 | — | — | 3.5 | — |
| 6′ | 165.8 | — | 2.3 | — | 4.5 | — | — |
| 7′ | 164.8 | 2.7 | — | — | — | — | — |
| 3-Me | 16.2 | — | 2.5 | — | — | — | 11.6 |
| 7-Me | 17.9 | — | 2.1 | — | — | — | 10.6 |
| 13-Me | 10.9 | — | 1.9 | — | — | — | — |
| 15-Me | 16.7 | — | 2.1 | — | — | — | 12.7 |
| 19-Me | 13.7 | — | 1.9 | — | — | — | 14.1 |
| 25-Me | 17.8 | — | 2.1 | — | — | — | 4.3 |
| 5′-Me | 10.2 | — | 2.6 | — | — | — | 15.3 |
| 23-OMe | 59.1 | — | — | 9.9[c] | — | — | — |

[a] Relative enrichments were normalized to peak intensities for the 23-OMe signal. [b] Enrichment ratio of the peak of tautomycin enriched by [1-$^{13}$C]isobutyrate was 5.2. [c] Relative enrichment of 23-OMe was normalized to peak intensity for the C-20 signal.

study, Ômura *et al.* also observed that sodium [1-$^{13}$C]acetate and [2-$^{13}$C]acetate were not incorporated into a $C_2$ unit corresponding to C-3 and -4 to which a hydroxyl and a methoxy group are attatched, respectively.[14] They postulated that the $C_2$ unit of leucomycin are derived from glycerol *via* glycine–glycolate pathway.[15] To test this possibility, a feeding experiment with [1,2-$^{13}C_2$]glycine which gives glyoxylate by transamination was performed. This $^{13}C$ double labelled unit was highly incorporated into C-22 and C-23 ($J_{CC}$ = 42.4 Hz) of tautomycin. This result indicated that glycine was incorporated

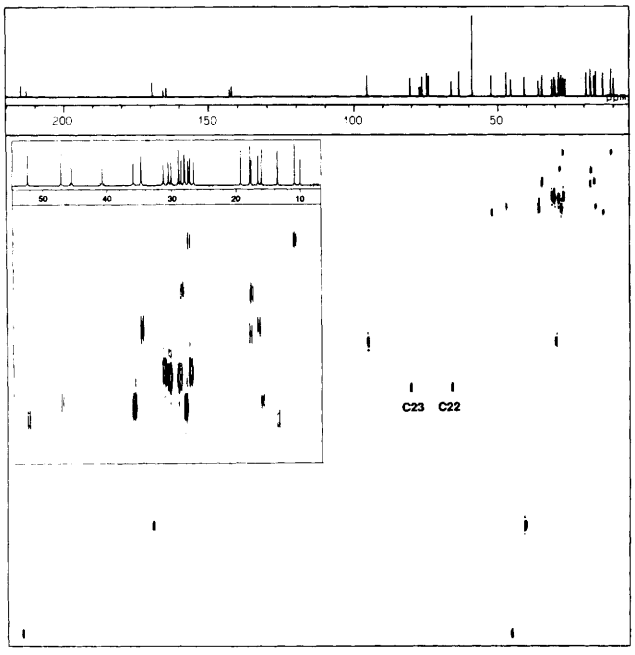



**Fig. 2** PFG 2-D INADEQUATE spectrum of tautomycin labelled with [1,2-$^{13}C_2$]glycine. JEOL α-400 FT NMR spectrometer, 35 mg 140 mm$^{-3}$ using micro NMR tube, $\tau$ = 5.8 ms, measurement time; 16 h.

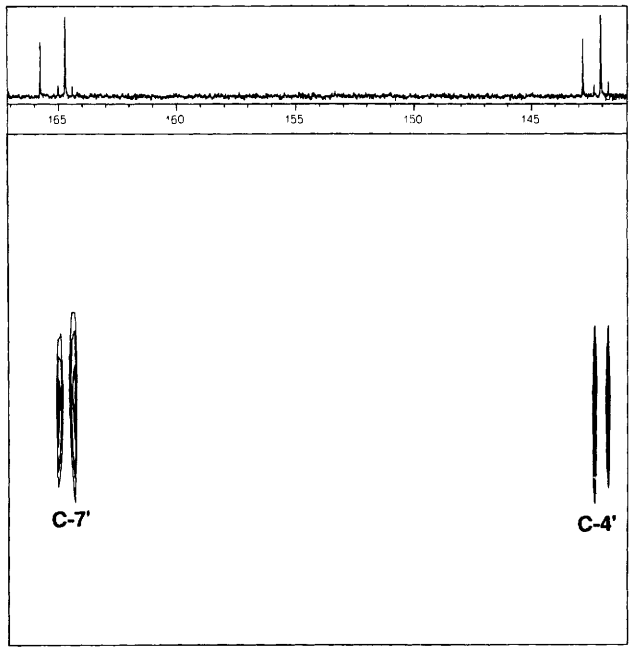



**Fig. 3** PFG 2-D INADEQUATE spectrum of tautomycin labelled with L-[1,2-$^{13}C_2$]glutamate. JEOL α-400 FT NMR spectrometer, 29 mg 140 mm$^{-3}$ using micro NMR tube, $\tau$ = 5.8 ms, measurement time; 22 h.

into C-22 and C-23 *via* the glyoxylate–glycolate pathway. The $^{13}C$-$^{13}C$ labelling pattern detected by the pulsed field gradient (PFG) 2-D INADEQUATE[16] technique using a micro NMR tube is shown in Fig. 2. [1,2-$^{13}C_2$]Glycine was also incorporated into six acetate units, C-4 and C-5, C-8 and C-9, C-10 and C-11, C-16 and C-17, C-20 and C-21, C-1′ and C-2′, probably through the glycine reductase pathway. However, the possibility of indirect incorporation of glycine *via* the glycine reductase pathway through acetate into C-22 and C-23 could be ruled out, because the higher enrichment at C-22 and C-23 were observed even in 1D $^{13}C$ NMR. The low level incorporation into [2,3-$^{13}C_2$]propionate units, C-3 and 3-Me, C-7 and 7-Me, C-13 and 13-Me, C-15 and 15-Me, C-19 and 19-Me, are reasonably explained by second cycle passage of acetate through the Krebs cycle followed by the methyl malonate–propionate shunt *via* succinate.[11]

On the basis of the above data, we conclude that the right half chain of the tautomycin molecule is synthesized by a polyketide pathway which starts with isobutyrate followed by introduction of a glycolate unit, and then five acetate and five propionate units. The terminal methyl carbon (C-1) was derived from [2-$^{13}C$]acetate which may be metabolized to β-keto carboxylic acid, after which the terminal methyl ketone is formed by decarboxylation.

The left half of the antibiotic, the dialkylmaleic anhydride moiety is synthesized from one propionate and a C-5 unit. Although the biosynthesis of 2-butyl-3-methylmaleic anhydride from the condensation of a hexanoyl derivative with oxalacetic acid has been reported,[17] there is no biosynthetic precedent for the 2-(1-hydroxy-2-carboxyethyl)-3-methylmaleic anhydride path. As we have described above, C-1′ and -7′ were derived from sodium [1-$^{13}C$]acetate and carbons 2′, 3′ and 4′ were derived from sodium [2-$^{13}C$]acetate. It is to be noted that the enrichment ratios at C-1′ and -2′ derived from sodium [1-$^{13}C$]acetate and [2-$^{13}C$]acetate are twice as high as those at C-7′ and C-4′. This labelling pattern suggests strongly that the C-5 unit may come from α-keto glutarate which is formed from acetate through Krebs cycle (Fig. 4). The clear evidence of this hypothesis was finally obtained from the feeding experiment of L-[1,2-$^{13}C_2$]glutamate which is a direct precursor of α-[1,2-$^{13}C_2$]keto glutarate (transamination). Only C-4′ and C-7′ ($J_{CC}$ 61.5 Hz) in tautomycin were enriched with L-[1,2-$^{13}C_2$]-glutamate. The PFG 2-D INADEQUATE spectrum of tautomycin labelled with L-[1,2-$^{13}C_2$]glutamate are shown in Fig. 3. This result directly evidenced that the C-5 unit was derived from α-keto glutarate. The dialkylmaleic anhydride moiety may be biosynthesized *via* an aldol type condensation of the ketone of α-keto glutarate with the active methylene of propionyl CoA, followed by dehydration and then hydration of the resulting allylic position at C-3′. Thus, the biosynthesis of tautomycin is depicted in Fig. 4. It is noteworthy that this experiment affords the first clear evidence of the biosynthesis of a C-5 unit which has been deduced from the distribution of label from $^{13}C$ acetate for the biosynthesis of domoic acid[18] and others.[19]

**Biosynthesis of tautomycetin**

Feeding experiments with sodium [1-$^{13}C$]acetate showed that carbons 4, 10, 14, 18, 1′ and 7′ were derived from sodium [1-$^{13}C$]acetate. Feeding experiments of sodium [2-$^{13}C$]acetate showed that carbons 1, 5, 6, 7, 8, 9, 11, 15, 19, 2′, 3′, 4′, 5′, 7-Me, 9-Me, 13-Me, 17-Me and 5′-Me were enriched. These results indicated randomization of [2-$^{13}C$]acetate to [1,2,3-$^{13}C_3$]-propionate as already observed and discussed with tautomycin. In the experiments using $^{13}C$ propionate, carbon 6, 8, 12, 16 and 6′ were derived from sodium [1-$^{13}C$]propionate and carbons 7-Me, 9-Me, 13-Me, 17-Me and 5′-Me were derived from sodium [3-$^{13}C$]propionate. These results indicated that five propionate units were incorporated into tautomycetin. In the case of sodium [2-$^{13}C$]propionate, $^{13}C$ enrichment at carbons 7, 9, 13, 17 and 5′ were observed. The experiment using sodium [1-$^{13}C$]butyrate showed that only the carbon 2 was derived from C-1 of butyrate (Table 2).

The $^{13}C$ NMR and Echo-type 2D INADEQUATE[13,20] spectra of tautomycetin labelled with [1,2-$^{13}C_2$]acetate are shown in Fig. 5. Five pairs of cross peaks observed between C-4 and C-5 ($J_{CC}$ 53.8 Hz), C-10 and C-11 ($J_{CC}$ 29.4 Hz), C-14 and

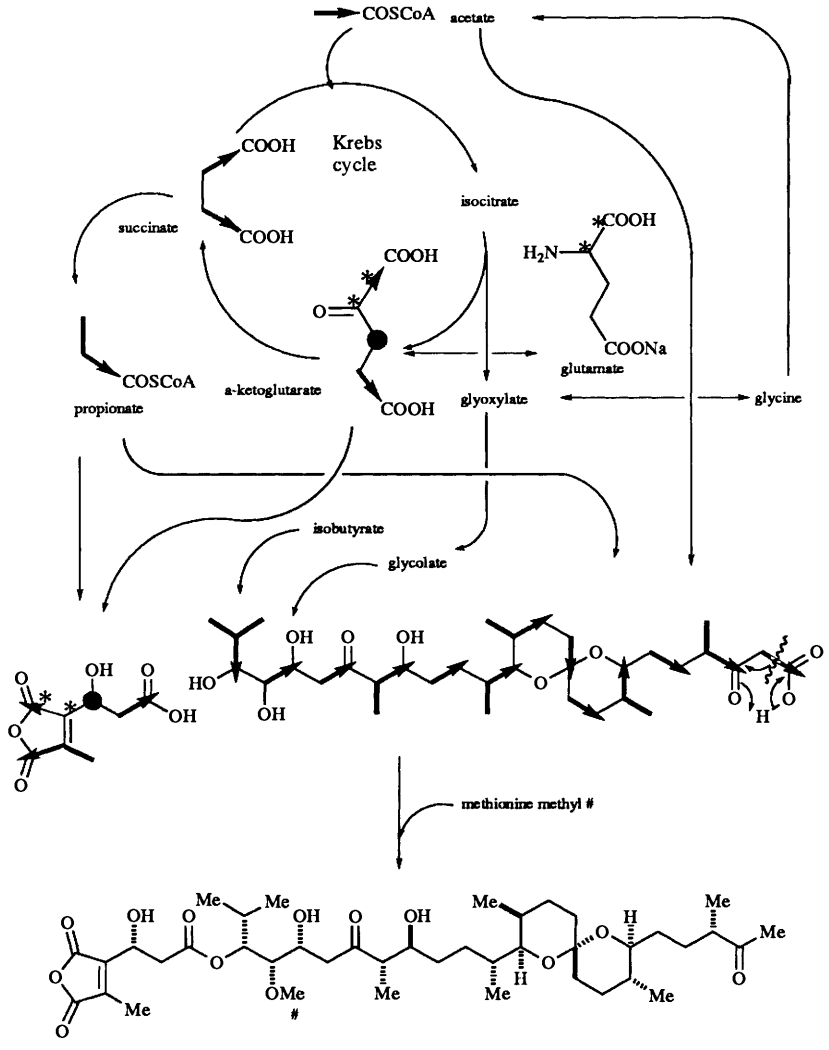



**Fig. 4** Biosynthesis of tautomycin. ●: derived from C-2 methyl carbon of [2-$^{13}$C]acetate. $^{13}$C Enrichment ratios at C-1′ and C-2′ are higher than those at C-4′ and C-7′.

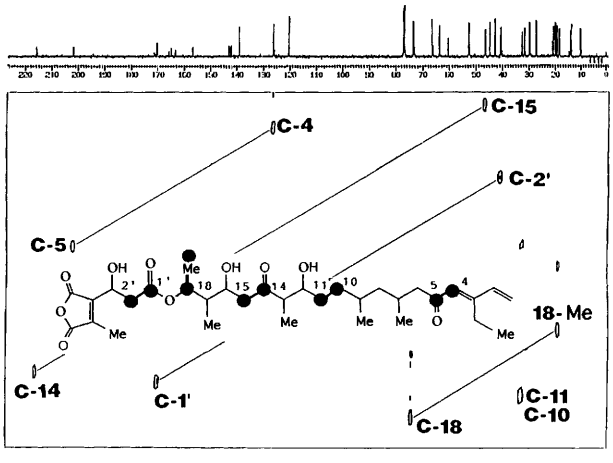



**Fig. 5** Echo-type 2D INADEQUATE spectrum of tautomycetin labelled with [1,2-$^{13}C_2$]acetate. JEOL GX-400 FT NMR spectrometer, $\tau$ = 5 ms, measurement time; 37 h.

C-15 ($J_{CC}$ 39.2 Hz), C-18 and 18-Me ($J_{CC}$ 41.9 Hz) and C-1′ and C-2′ ($J_{CC}$ 57.4 Hz) confirmed the five acetate units. No radomization occurred and cross peaks between C-4′ and C-7′ could not be detected in this case.

On the basis of the above data, the biosynthetic origin of tautomycetin can be summarized as follows. The right half chain of the tautomycetin molecule is formed *via* the polyketide pathway which starts with acetate followed by introduction of three acetate and four propionate units and one butyrate unit. The terminal methyl carbon (C-1) was derived from [2-$^{13}$C]acetate. The data suggests that the terminal methylene are formed by decarboxylation of the corresponding β-keto carboxylate or β-hydroxy carboxylate anion. The left half dialkylmaleic anhydride moiety has the same structure as that of tautomycin and gave a similar enrichment pattern. As described above, C-1′ and -7′ were derived from [1-$^{13}$C]acetate and C-2′, -3′ and -4′ were derived from [2-$^{13}$C]acetate. This labelling pattern suggested that the anhydride moiety was formed by condensation of one molecule of propionate with the C-5 unit derived from α-keto glutarate as in the case of tautomycin (Fig. 6). Since the absolute configuration of the dialkylmaleic anhydride moiety of tautomycetin is identical with that of tautomycin,† both the moieties may be biosynthesized by similar enzyme systems.

† CD curve of the trimethyl ester derived from the dialkylmaleic anhydride moiety of tautomycin was identical with that of tautomycetin.

**Table 2** $^{13}C$ Chemical shifts and isotopic incorporation into tautomycetin

| Carbon No. | $\delta(^{13}C)$ | Relative enrichment[a] [1-$^{13}$C]-Acetate | [2-$^{13}$C]-Acetate | [1-$^{13}$C]-Butyrate | [1-$^{13}$C]-Propionate | [2-$^{13}$C]-Propionate | [3-$^{13}$C]-Propionate |
|---|---|---|---|---|---|---|---|
| 1 | 120.0 | — | 1.6 | — | — | — | — |
| 2 | 139.0 | — | — | 9.0 | — | — | — |
| 3 | 156.0 | — | — | — | — | — | — |
| 4 | 126.0 | 1.6 | — | — | — | — | — |
| 5 | 201.0 | — | 1.9 | — | — | — | — |
| 6 | 52.8 | — | 1.8 | — | 13.4 | — | — |
| 7 | 27.1 | — | 1.4 | — | — | 6.3 | — |
| 8 | 44.9 | — | 1.4 | — | 13.1 | — | — |
| 9 | 29.8 | — | 1.5 | — | — | 6.5 | — |
| 10 | 32.5 | 1.7 | — | — | — | — | — |
| 11 | 31.7 | — | 1.6 | — | — | — | — |
| 12 | 73.6 | — | 1.6 | — | 10.7 | — | — |
| 13 | 52.5 | — | 1.8 | — | — | 4.2 | — |
| 14 | 215.0 | 1.7 | — | — | — | — | — |
| 15 | 46.6 | — | 1.4 | — | — | — | — |
| 16 | 66.0 | — | — | — | 11.2 | — | — |
| 17 | 42.8 | — | 1.3 | — | — | 4.9 | — |
| 18 | 73.4 | 1.5 | — | — | — | — | — |
| 19 | 18.4 | — | 1.4 | — | — | — | — |
| 1′ | 170.0 | 2.1 | — | — | — | — | — |
| 2′ | 40.6 | — | 1.3 | — | — | — | — |
| 3′ | 63.0 | — | 1.3 | — | — | — | — |
| 4′ | 142.2 | — | 1.7 | — | — | — | — |
| 5′ | 142.9 | — | 1.5 | — | — | 5.1 | — |
| 6′ | 165.7 | — | — | — | 14.3 | — | — |
| 7′ | 164.8 | 1.6 | — | — | — | — | — |
| 7-Me | 20.0 | — | 1.4 | — | — | — | 3.6 |
| 9-Me | 19.2 | — | 1.5 | — | — | — | 4.9 |
| 13-Me | 28.7 | — | 1.4 | — | — | — | 4.0 |
| 17-Me | 10.3 | — | 2.0 | — | — | — | 5.2 |
| 5′-Me | 10.2 | — | 2.0 | — | — | — | 5.1 |
| 1″ | 20.5 | — | — | — | — | — | — |
| 2″ | 13.9 | — | — | — | — | — | — |

[a] Relative enrichments were normalized to peak intensities for the C-2″ signal.

## Experimental

$^{13}C$ NMR spectra were measured on a JEOL FX-100 FT NMR spectrometer. 2D INADEQUATE spectra were recorded on JEOL GX-400 FT NMR. Pulsed field gradient (PFG) 2D-INADEQUATE spectra were recorded on a JEOL α-400 FT NMR spectrometer with a PFG unit. Micro NMR tubes were made by Shigemi Ltd, Japan. The $^{13}C$ labelled acetate and propionate (90 atom%) were purchased from MSD ISOTOPES, Canada. [1-$^{13}$C]Butyrate (99 atom%) was purchased from ICON, U.S.A. L-[*methyl*-$^{13}$C]Methionine (98.2 atom%) was purchased from MSD ISOTOPES, Canada. [1,2-$^{13}C_2$]Glycine (99 atom%) was purchased from ISOTEC, U.S.A. L-[1,2-$^{13}C_2$]Glutamic acid (99 atom%) was purchased from EURISO-TOP, France.

### Culture of the $^{13}C$-labelled tautomycin-producing strain

*Streptomyces spiroverticillatus* sp. JC-84-44 grown on starch-yeast agar was inoculated into a 400 $cm^3$ cylindrical flask containing 70 $cm^3$ of a medium composed of glucose 2%, soluble starch 1%, meat extract 0.1%, dry yeast 0.4%, soybean flour 2.5%, NaCl 0.25% and $K_2HPO_4$ 0.005%, and cultured at 28 °C for 48 h on a rotary shaker. Aliquots (0.5 $cm^3$) of the seed culture was inoculated into the same medium. Fermentation was carried out on a rotary shaker at 28 °C. After 24 h of fermentation, a $^{13}C$-labelled precursor was added to the culture at a concentration of 0.6% (w/v), and fermentation was continued for an additional 48 h.

In the cases of [1,2-$^{13}C_2$]glycine and L-sodium [1,2-$^{13}C_2$]glutamate prepared from L-[1,2-$^{13}C_2$]glutamic acid neutralized with 0.1 mol dm-$^{13}$ aq. NaOH, precursors were added in doses of 0.007% (w/v) at 24, 30, 36 and 46 h after inoculation, and the cultures were further incubated for 24 h.

### Isolation of $^{13}C$-labelled tautomycin

Each fermentation broth (490 $cm^3$; pH 7.2; 350 $cm^3$ in the cases of [1,2-$^{13}C_2$]glycine and L-sodium [1,2-$^{13}C_2$]glutamate) was filtered; the filtrate was extracted with EtOAc and the mycelium cake was extracted with acetone. The acetone extract was concentrated under reduced pressure to give an aqueous solution, which was then extracted with EtOAc. Both the EtOAc extracts were combined and evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (4:1). The eluate was monitored with silica gel TLC, and then further purified by reverse phase HPLC [Senshu pak ODS-H column with MeOH–$H_2O$–buffer (1% diethylamine–formic acid, pH 7.3), 8:1:1 as a solvent system]. The fraction were adjusted to pH 4 with 1 mol $dm^{-3}$ HCl and concentrated to give an aqueous solution, which was then extracted with EtOAc and evaporated under reduced pressure to give pure tautomycin. From each 490 $cm^3$ of the culture broth supplemented with $^{13}C$-labelled precursors, 20–45 mg of purified $^{13}C$-labelled tautomycin was obtained. The yields of [1,2-$^{13}C_2$]glycine labelled and L-[1,2-$^{13}C_2$]glutamate labelled tautomycins were 35.7 mg and 29.9 mg, respectively.

### Culture of $^{13}C$ labelled tautomycetin-producing strain and isolation of $^{13}C$ labelled tautomycetin

For preparation of $^{13}C$ labelled tautomycetin, *Streptomyces griseochromogenus* sp. JC-84-1223 was used. The cultural

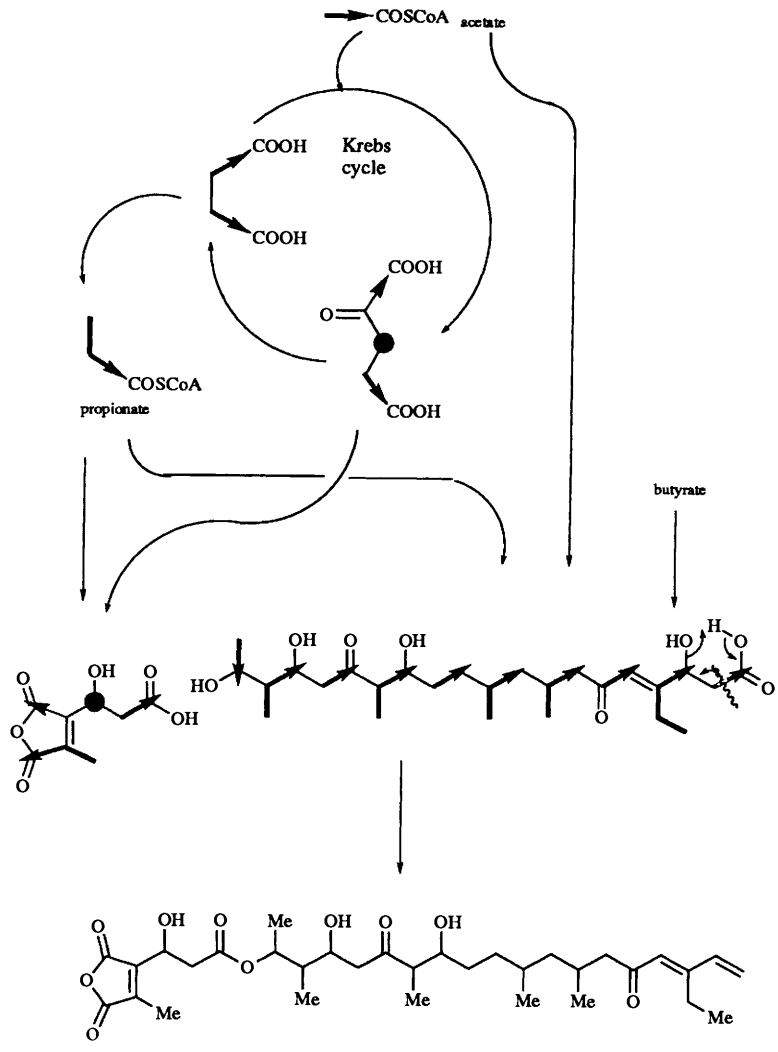



**Fig. 6** Biosynthesis of tautomycetin. ●: derived from C-2 methyl carbon of [2-$^{13}$C]acetate.

conditions and isolation procedure were the same as those for $^{13}$C labelled tautomycin, except that the volume of the fermentation broth of tautomycetin was increased to 980 cm$^3$ and that MeOH–buffer for HPLC was adjusted to pH 4.0.

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