Synthesis, characterization, and enzymic conversion of nonhydrolysable analogues of propionylcoenzyme A

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This paper is dedicated to Professor Ian D. Spenser

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We describe the synthesis of three novel analogues of propionyl-coenzyme A, in which the sulfur atom has been replaced by methylene, ethylene, and thiomethylene, respectively. All three analogues, propionyl-dethia(carba)-CoA (**1**), propionyl-dethia-(dicarba)-CoA (**2**), and *S*-(2-oxobutanyl)-CoA (**3**) were characterized by ^1H and ^{31}P NMR spectroscopy and FAB mass spectrometry. Propionyl-CoA–oxaloacetate transcarboxylase from *Propionibacterium shermanii* accepted the novel analogues as substrates and carboxylated them to the corresponding methylmalonyl-CoA analogues (**4–6**). The latter were further converted into the succinyl-CoA analogues by the coenzyme-B_{12}-dependent methylmalonyl-CoA mutase from the same organism. The succinyl-CoA analogues, succinyl-dethia(carba)-CoA (**7**), succinyl-dethia(dicarba)-CoA (**8**), and 4-carboxy(2-oxobutanyl)-CoA (**9**) were obtained on a preparative scale and their Michaelis constants (K_m) with methylmalonyl-CoA mutase were determined to be 0.136, 2.20, and 0.132 mM, respectively (K_m for succinyl-CoA is 0.025 mM). The V_{max} values for **7**, **8**, and **9** are 1.1, 0.013, and 0.0047 μmol min^{-1} U^{-1}, respectively (V_{max} for succinyl CoA is 1.0). The utility of the novel coenzyme A analogues in enzyme mechanistic studies is discussed.

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On décrit la synthèse de trois nouveaux analogues du propionyl-coenzyme- A dans lesquels l'atome de soufre a été remplacé respectivement par un méthylène, un éthylène et un thiométhylène. On a caractérisé les trois analogues, le propionyl-déthia(carba)-CoA (**1**), le propionyl-déthia(dicarba)-CoA (**2**) et le *S*-(2-oxo-butanyl)-CoA (**3**) par spectroscopie RMN du ^1H et du ^{31}P et par spectrométrie de masse FAB. La propionyl-CoA–oxaloacétate transcarboxylase du *Propionibacterium shermanii* accepte les nouveaux analogues comme substrats et par carboxylation les transforme en analogues méthylmalonyl- CoA correspondants (**4–6**). Par la suite, on a transformé ces derniers en analogues succinyl-CoA en les soumettant à la méthylmalonyl-CoA mutase dépendante du coenzyme-B_{12} provenant du même organisme. On a obtenu les analogues succinyl-CoA, succinyl-déthia(carba)-CoA (**7**), succinyl-déthia(dicarba)-CoA (**8**) et 4-carboxy(2-oxobutanyl)-CoA (**9**) à une échelle préparative et on a déterminé que leurs constantes de Michaelis (K_m) avec la méthylmalonyl-CoA mutase sont respectivement 0,136, 2,20 et 0,132 mM (K_m de la succinyl-CoA est égale à 0,025 mM). Les valeurs de V_{max} pour les composés **7**, **8** et **9** sont respectivement 1,1, 0,013 et 0,0047 μmol min^{-1} U^{-1} (V_{max} pour le succinyl-CoA est égal à 1,0). On discute de l'utilité des nouveaux analogues du coenzyme A dans des études mécanistiques d'enzymes.

[Traduit par la rédaction]

Introduction

Coenzyme A esters play important roles as substrates or regulators of a great number of enzymic reactions including carboxylation–decarboxylations, Claisen-type or aldol-type condensations, thioclastic cleavage, reductions, dehydrogenations, hydration–dehydrations, acylations, isomerizations, and epimerizations. While in some of these reactions the thiolester bond is cleaved, in others it is retained in the product(s). Under neutral and alkaline conditions thiolesters can spontaneously hydrolyse, which may complicate mechanistic studies. In 1978 Stewart and Wieland (1) synthesized a nonhydrolysable analogue of acetyl-CoA, acetonyldethia-CoA (acetyl-CH_2CoA), in which the sulfur had been replaced by methylene. This analogue turned out to be a powerful inhibitor of some enzymic reactions (1, 2), and an allosteric effector of others (2). It was a good substrate for the biotin-enzyme acetyl-CoA carboxylase (3).

Following our interest in the mechanism of propionic acid metabolism, we prepared three different analogues of propionyl-CoA, namely propionyl-dethia(carba)-CoA **1**, propionyl-dethia(dicarba)-CoA **2**, and 2oxobutanyl-CoA **3**. We describe here the syntheses and spectroscopic characterization of these three analogues, as well as their behaviour in some enzymic reactions. A short communication on compound **1** appeared earlier (4).

Results

*Synthesis of propionyl-dethia(carba)-coenzyme A(**1**)*

Following the strategy applied for the total synthesis of coenzyme A (5) and of acetyldethia(carba)CoA (1), the preparation of two key intermediates, propionyl-dethia(carba)pantetheine-4-phosphate (**10**) and adenosine-2′,3′ cyclophosphate 5′ phosphomorpholidate (**11**), was envisaged (Scheme 1). Michael addition of nitromethane to ethylvinylketone gave 1-nitro-4-oxo-hexane (**12**) in 50% yield. After protection of the keto function as the 1,3-dioxolane (**13**, 80%), the nitro group was reduced with Raney nickel to furnish 2-(3-aminopropyl)-2-ethyl-1,3-dioxolane (**14**) in 82% yield. Condensation of the latter with 4-nitrophenyl benzyloxycarbonyl-β-alanine (**15**) (6, 7) resulted in 2-[3-(*N*-benzyloxycarbonyl-β-alanyl)-aminopropyl]-2-ethyl-1,3-dioxolane (**16**, 64.5%). Removal of the *N*-benzyloxycarbonyl protecting group by catalytic hydrogenation was followed, without isolating the intermediate **17**, by condensation in the melt with (−)(*R*)-pantolactone. Two subsequent filtrations, first through a Dowex 50 W × 4 (H^+), then through a Dowex 2 × 8 (^-OH) column, removed the protecting ketal group and afforded propionyl-dethia(carba)pantetheine **18** in 50% yield (based on **16**). Phosphorylation of **19** was carried out with cyanoethylphosphate in analogy to the procedure of Stewart and Wieland (1) and yielded propionyl-dethia(carba)pantetheine-

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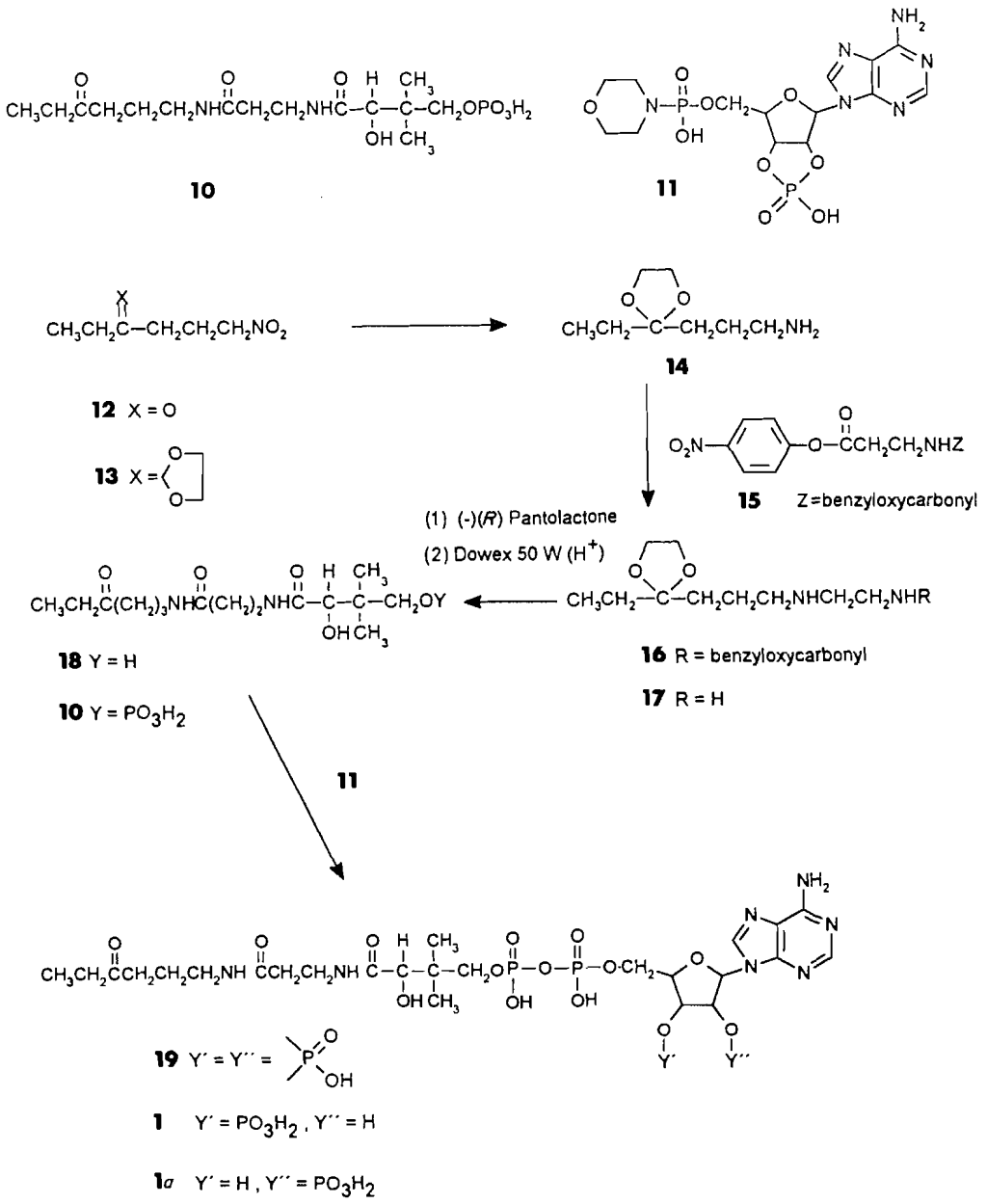


SCHEME 1

4-phosphate (**10**, 42%). Adenosine-2′,3′-cyclophosphate-5′-phosphomorpholi- date (**11**) was prepared according to one of the methods described by M. Yoshikawa et al. (8), Nikawa et al. (3), or A. Simoncsits and J. Tomasz (9). Compound **11** was first described by Moffat and Khorana (5).

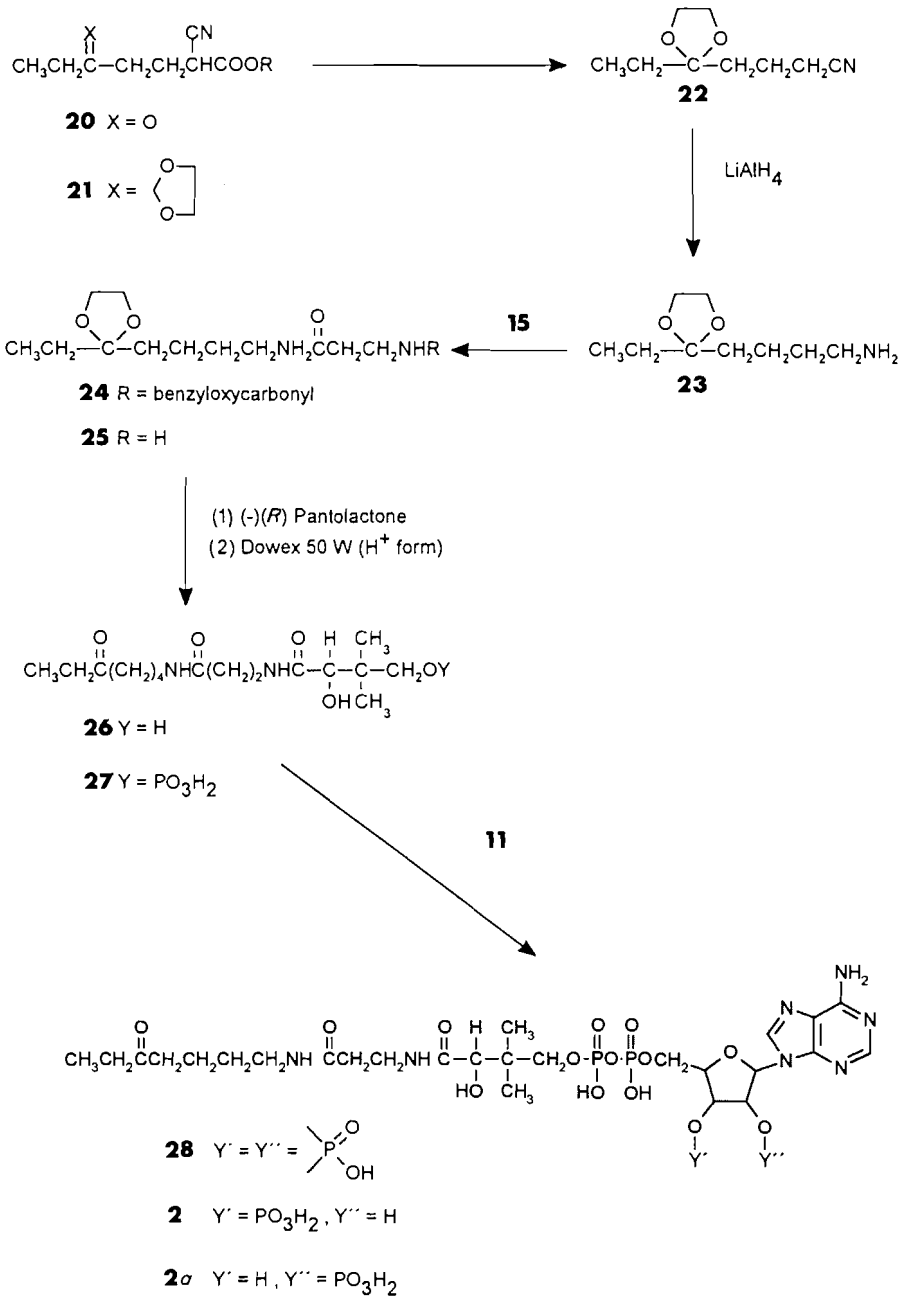
Having the two key intermediates **10** and **11** in hand, their condensation to the coenzyme-A analogue could be realized. Acid hydrolysis of the product (**19**) led to a mixture (41%), which was partially separated to propionyl-CH_2CoA (**1**) and propionyl-CH_2-isoCoA (**1***a*) by DEAE cellulose chromatography using a linear LiCl gradient. The final preparation of **1** still contained ~9% **1***a* and was charaterized by ^{1}H and ^{31}P NMR spectroscopy.

Synthesis of propionyl-dethia(dicarba)-coenzyme A **2**

Except for the initial steps, the same method was used as described for analogue **1** (Scheme 2). In the first step the anion of cyanoacetic acid ethylester was added to ethylvinylketone to yield 2-cyano-5-oxoheptanoic acid ethylester (35%). Protection of the keto group afforded 2-(3-cyano-3-carbethoxyethyl)-2-ethyldioxolane (**21**) (83%). The carbethoxy group was removed by heating **21** in a solution of sodium chloride in dimethyl sulfoxide and water to furnish 2-(3-cyanopropyl)-2-ethyldioxolane (**22**) in 69% yield. Reduction of **22** with $LiAlH_4$ gave 2-(4-aminobutyl)-2-ethyldioxolane (**23**) (76%), which was treated further as described for its lower homologue (**14**). This analogous synthesis yielded, after chromatographic separation, propionyl-CH_2CH_2.CoA (**2**) and propionyl-CH_2CH_2-isoCoA (**2***a*) in similar quantities. Both were characterized by ^{1}H and ^{31}P NMR spectroscopy and **2** also by FAB mass spectrometry.

Synthesis of S*-(2-oxobutyl)-coenzyme A (3)*

Compound **3** was synthesized by reaction of coenzyme A



SCHEME 2

with an excess of 1-bromo-2-butanone. After work-up and chromatography as described for analogues **1** and **2** the product was characterized by FAB mass spectrometry and ^{1}H and ^{31}P NMR spectroscopy. Both measurements were consistent with the anticipated structure **3** (see Experimental).

*Conversion and kinetic behaviour of the propionyl-coenzyme A analogues **1**, **2**, and **3** with transcarboxylase*

Propionyl-CH_2-CoA **1**, propionyl-CH_2CH_2 CoA **2**, and *S*-(2-oxobutyl)-CoA **3** were carboxylated to the corresponding methylmalonyl derivatives **4**, **5**, and **6** by incubation with propionyl-CoA–oxaloacetate transcarboxylase from *Propionibacterium shermanii* in the presence of oxaloacetate, lactate dehydrogenase, and NADH. The reaction is summarized in eq. [1]

[1] propionyl-X-CoA + oxaloacetate + NADH + H^+ $\xrightarrow[\text{lactate dehydrogenase}]{\text{transcarboxylase}}$ methylmalonyl-X-CoA + lactate + NAD^+

Additions of lactate dehydrogenase and NADH were necessary to push the equilibrium to the right. After precipitation of the products with acetone, the centrifuged pellets were dried, dissolved in deuterium oxide, and their ^{1}H NMR spectra recorded. All signals could be assigned to the expected structures (see Experimental). Upon prolonged incubation in deuterated phosphate buffer ($p^2H = 7.1$) the methyl doublet at 1.20 ppm slowly changed and was eventually converted into a singlet. This was a measure for the $H/^2H$ exchange rate of the α proton of the methylmalonyl moiety.

TABLE 1. Comparison of the K_m and V_{max} values of propionyl-S-CoA and its analogues **1**, **2**, and **3** and in the transcarboxylase reaction

Substrate	K_m (mM)	V_{max} (μmol min^{-1} U^{-1})
$CH_3CH_2\overset{O}{\overset{\|}{C}}S\text{-CoA}$	0.047	1
$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_2\text{-CoA}$	0.15	0.5
$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_2CH_2\text{-CoA}$	0.14	0.025
$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_2S\text{-CoA}$	0.44	0.020

TABLE 2. Comparison of the K_m and V_{max} values of succinyl-S-CoA and its analogues **7**, **8**, and **9** in the methylmalonyl-CoA mutase reaction and the length of X in succinyl-X-CoA (*d*)

Substrate	*d* (Å)	K_m (mM)	V_{max} (μmol min^{-1} U^{-1})
$HOOCCH_2CH_2\overset{O}{\overset{\|}{C}}S\text{-CoA}$	2.75	0.025	1.0
$HOOCCH_2CH_2\overset{O}{\overset{\|}{C}}CH_2\text{-CoA}$	2.48	0.136	1.1
$HOOCCH_2CH_2\overset{O}{\overset{\|}{C}}CH_2CH_2\text{-CoA}$	3.83	2.20	0.013
$HOOCCH_2CH_2\overset{O}{\overset{\|}{C}}CH_2S\text{-CoA}$	4.16	0.132	0.0047

The kinetic measurements with **1**, **2**, and **3** were carried out according to the published assay method (11). The K_m and V_{max} values were determined by the method of Lineweaver–Burk (11) and are listed in Table 1.

*Conversion of the propionyl-coenzyme A analogues **1**, **2**, and **3** into the corresponding succinyl derivatives **7**, **8**, and **9** with an enzyme cocktail*

On an analytical scale, the reaction sequence was monitored directly in the NMR tube. In separate experiments each of the analogues, i.e., propionyl-CH_2-CoA **1**, propionyl-CH_2CH_2-CoA **2**, or *S*-(2-oxobutanyl)-CoA **3**, was dissolved in deuterated phosphate buffer. The reaction was initiated by addition of a lyophilized enzyme mixture consisting of methylmalonyl-CoA mutase, epimerase, and transcarboxylase (12) (eq. [2]).

[2] propionyl-X-CoA + oxaloacetate $\xrightarrow[\text{epimerase, mutase}]{\text{transcarboxylase}}$ succinyl-X-CoA + pyruvate

Conversions of the analogues to their succinyl derivatives could be followed by ^1H NMR spectroscopy. On a preparative scale, the same reactions were carried out in aqueous (nondeuterated) phosphate buffer. After chromatography of the products on a DE-32 column, essentially pure succinyl-CoA analogues, i.e., succinyl-CH_2-CoA (**7**), succinyl-CH_2CH_2-CoA (**8**), and *S*-(2-oxo-4-carboxybutyl)-CoA (**9**), were obtained and characterized by ^1H and ^{31}P NMR spectroscopy.

The K_m and V_{max} values of **7**, **8**, and **9**, as substrates of methylmalonyl-CoA mutase, were determined by the usual spectrophotometrical assay (10, 12) and by using the method of Lineweaver–Burk (11). The results are summarized in Table 2.

Discussion

The synthesis of the novel propionyl-CoA analogues **1**, **2**, and **3** was carried out by conventional methods. While in the case of **1** and **2** a strategy similar to that used for the total synthesis of coenzyme A (5) was applied (Schemes 1, 2), **3** was available by a much simpler procedure involving the reaction of 1-brom-2-butanone with commercial coenzyme A.

Comparison of the geometry of the new analogues with that of natural propionyl-S-CoA is of interest (Table 2). Whereas the C—C bond is shorter than the C—S bond, in propionyl-CH_2CH_2-CoA **2** we have a slightly longer and in propionyl-CH_2-S-CoA **3** a substantially longer CoA chain, respectively.

It is noteworthy that all three propionyl-CoA analogues are substrates of the propionyl-CoA–oxaloacetate transcarboxylase (Table 1). Since acetyl-CH_2CoA was carboxylated by acetyl-CoA carboxylase (3) it was not surprising that its propionyl homologue **1** was also a transcarboxylase substrate. The longer CoA chain has a relatively small effect on K_m but a larger one on V_{max}. In all cases it is possible to carry out the enzymic carboxylation on a preparative scale and to spectroscopically characterize the products.

Prolonged observation of the ^1H NMR spectrum of **1** permitted determination of the spontaneous $^1H/^2H$ exchange rate of the α proton in the methylmalonyl moiety. With a half lifetime of 140 min at p^2H^2 7.1, it was somewhat faster than the value of $t/2 = 480$ min at p^2H^2 7.1 reported for methylmalonyl-thiolester (13).

The enzymically produced methylmalonyl-CoA analogues **4**, **5**, and **6** were also accepted as substrates by the coenzyme B_{12}-dependent methylmalonyl-CoA mutase. In the presence of the transcarboxylase, the epimerase, and the mutase, the propionyl-CoA analogues **1**, **2**, and **3** were converted in one pot into the corresponding succinyl-CoA analogues **7**, **8**, and **9** on a preparative scale. These nonhydrolysable succinyl derivatives were used as substrates to determine the kinetic constants of the mutase reaction (Table 2).

A comparison of the K_m values reveals that both the length of the CoA chain and the presence of sulfur is important. Succinyl-CH_2CH_2-CoA (**8**) has the lowest affinity for the mutase, while succinyl-CH_2S-CoA has only a slightly higher K_m than succinyl-S-CoA. Interestingly, the shorter the CoA chain, the higher the V_{max} value.

The novel, nonhydrolysable, propionyl, methylmalonyl, and succinyl-CoA analogues may be useful in mechanistic and structural studies of the corresponding enzymes by ESR, NMR, and CD spectroscopy as well as by X-ray crystallography.

Experimental

Equipment

^1H NMR, ^{31}P NMR, mass and IR spectra were recorded with the following instruments: Bruker WH 250 or AM 400, Bruker WH 300,

[2]The p^2H was determined in deuterium oxide.

Varian MAT CH-5 or Finnigan MAT 90, and Beckman IR-8, respectively.

For enzyme assays a Perkin Elmer 550A (UV–vis) spectrometer was used. Melting points were determined in a Büchi apparatus according to Dr. Tottoli.

For HPLC separations a Merck–Hitachi instrument was used.

Materials

Ethylvinylketone, cyanoethylphosphate, β-alanine, (−)-(*R*)-pantolactone, phosphorus oxychloride, and coenzyme B_{12} were products of Fluka (Buchs, Switzerland). Trimetaphosphate, 5′-AMP, and 2′(3′)-AMP were from Sigma (St. Louis, U.S.A.), and DE-32 cellulose from Whatman (Maidstone, England). Coenzyme A, lactate dehydrogenase, NAD^+, and NADH were from Boehringer (Mannheim, Germany). Methylmalonyl-CoA mutase, propionyl-CoA–oxaloacetate transcarboxylase and methylmalonyl-CoA epimerase were isolated from *Propionibacterium shermanii* (10, 12).

Syntheses

1-Nitro-4-oxo-hexane (12) was synthesized as described for the 5-methyl derivative (14): 1 g (11.9 mmol) ethyl vinyl ketone yielded 0.87 g (5.95 mmol) **12** (50%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.06 (t, J = 8.3 Hz, 3H), 2.26 (quint, J = 7.8 Hz, 2H), (quart, J = 8.3 Hz, 2H), 2.58 (t, J = 7.8 Hz, 2H), 4.43 (t, J = 7.8 Hz, 2H).

*2-(3-Nitropropyl-)2-ethyl-1,3-dioxolane (**13**)* was obtained in analogy to the 2-isopropyl derivative (14); 0.66 g (4.5 mmol) **12** yielded 0.8 g (3.57 mmol) **13** (80%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.91 (t, J = 8.3 Hz, 3H), 1.65 (quart, J = 8.3 Hz, 2H), 1.73 (m, 2H), 2.11 (quint, J = 7.5 Hz, 2H), 3.95 (s, 4H), 4.41 (t, J = 7.5 Hz, 2H).

*2-(3-Aminopropyl-2-ethyl-1,3-dioxolane (**14**)* was synthesized as described for the 2-isopropyl derivative (14). Starting from 5.75 g (30.5 mmol) **13** we obtained 4.0 g (25 mmol) **14** (82%). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.93 (t, J = 8.3 Hz, 3H), 1.35 (s, 2H), 1.55 and 1.68 (m, 6H), 2.70 (t, J = 7.8 Hz, 2H), 3.98 (s, 4H).

*2-(3-[(N-Benzyloxycarbonyl-β-alanine)-aminopropyl)-2-ethyl-1,3-dioxolane (**16**)* was obtained in analogy to the 2-isopropyl derivative (14). Reaction of 1 g (6.28 mmol) **14** with 2.18 g (6.3 mmol) 4-nitrophenyl benzyloxycarbonyl-β-alanine (**15**) afforded 1.47 g (4.06 mmol) **16** (64.5%); mp 56–57°C; IR (KBr): 3320 (s), 3080 (w), 2980 (s), 2960 (s), 2880 (m), 1685 (s), 1635 (s), 1545 (s), 1460 (w), 1430 (w), 1370 (w), 1335 (m), 1265 (m), 1240 (m), 1190 (w), 1160 (w), 1135 (w), 1070 (m), 910 (w), 750 (w), 690 (m) cm^{-1}. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.91 (t, J = 8.3 Hz, 3H), 1.65 (m, 6H), 2.41 (t, J = 5.85 Hz, 2H), 3.26 (quart, J = 6.4 Hz, 2H), 3.5 (quart, J = 5.85 Hz, 2H), 3.95 (s, 4H), 5.08 (s, 2H), 5.46 (s, 1H), 5.78 (s, 1H), 7.33 (s, 5H), MS (T_d 130°C, 70 eV, *m/e*): 56 (14%), 57 (62), 65 (10), 69 (17), 70 (10), 73 (23), 77 (22), 79 (43), 86 (42), 91 (85), 96 (10), 97 (13), 98 (19), 99 (23), 101 (100), 102 (15), 107 (29), 108 (37), 113 (18), 130 (49), 139 (10), 142 (23), 227 (31), 335 (11), M^+ = 364 (6). Anal. calcd.: C 62.6, H 7.74, N 7.68; found: C 62.2, H 7.96, N 7.74%.

*Propionyl-dethia(carba)pantetheine (**18**)* was synthesized as described for the isobutanoyl derivative (14): 0.854 g (2.34 mmol) **16** yielded 370 mg (1.16 mmol) **18** (50%). IR (film): 3320 (s), 3100 (w), 2990 (s), 2970 (s), 1710 (s), 1650 (s), 1640 (s), 1570 (s), 1550 (s), 1530 (s), 1470 (m), 1440 (m), 1380 (m), 1360 (m), 1300 (m), 1250 (w), 1080 (m), 1050 (m), 880 (w) cm^{-1}. ^{1}H NMR (D_2O, 250 MHz) δ: 0.78 (s, 3H), 0.81 (s, 3H), 0.90 (t, J = 7.9 Hz, 3H), 1.63 (quint, J = 7.7 Hz, 2H), 2.4 (m, 6H), 3.05 (t, J = 7.7 Hz, 2H), 3.36 (AB system, J = 12 Hz, 2H), 3.41 (m, 2H), 3.86 (s, 1H). MS (T_d 160°C, 70 eV, *m/e*): 57 (43%), 58 (13), 71 (100), 72 (24), 86 (24), 98 (22), 99 (30), 100 (13), 115 (26), 116 (26), 157 (13), M^+ 1 = 317 (<1%).

*Propionyl-dethia(carba)pantetheine-4′-phosphate (**10**)* was obtained as described for the isobutanyl derivative (14). Reacting 637 mg (1.98 mmol) **18** with 1 g (2.63 mmol) cyanoethylphosphate (6) we obtained 470 mg (0.83 mmol) **10** (42%). IR (KBr): 3360, (s), 2940 (m), 1710 (m), 1655 (s), 1545 (m), 1450 (w), 1380 (w) 1245 (w), 1080 (s), 990 (s) cm^{-1}. ^{1}H NMR (D_2O, 250 MHz) δ: 0.83 (s, 3H), 0.98 (s, 3H), 1.0 (t, J = 9 Hz, 3H), 1.73 (quint, J = 6.5 Hz, 2H), 2.5 (m, 6H), 3.16 (t, J = 6.5 Hz, 2H), 3.52 (t, J = 5.5 Hz, 2H), 3.74 (m, 2H), 4.10 (s, 1H). ^{31}P NMR (D_2O, H_3PO_4 ext.) δ: 6.2 (s).

*Propionyl-dethia(carba) coenzyme A (**1**)* was prepared as described for its isobutanoyl analogue (14). Starting from 141.5 mg (0.25 mmol) **10** and 298.4 mg (0.62 mmol) adenosine-2′,3′ cyclophosphate-5′-phosphomorpholidate (**11**) we obtained a 48 mg (0.05 mmol) mixture of **1** and propionyl-dethia(carba) isocoenzyme A (**1*a***); 23 mg of this mixture was rechromatographed on a DE 32 cellulose column (2.2 ×30 cm) with an LiCl gradient (14). The first eluted peak contained **1*a*** (8.0 mg, 8.3 μmol) while **1** appeared in the second peak (8.3 mg, 8.6 μmol). ^{1}H NMR of **1** (D_2O, pD = 3, 250 MHz) δ: 0.60 (s, 3H), 0.70 (s, 3H), 0.83 (t, J = 6.8 Hz, 3H), 1.51 (quint, J = 6.6 Hz, 2H), 2.32 (m, 6H), 2.91 (t, J = 6.6 Hz, 2H), 3.30 (t, J = 7.0 Hz, 2H), 3.60 (ABX system, J = 12 Hz, J = 5 Hz, 2H), 3.90 (s, 1H), 4.1 (m, 2H), 6.09 (d, J = 8.3 Hz, 1H), 8.18 (s, 1H), 8.43 (s, 1H). ^{31}P NMR (D_2O, 121.48 MHz, H_3PO_4 ext.) δ: = −7.89 (m), 2.7 (s).

*2-(3-Cyano-3-carbethoxyethyl)-2-ethyldioxolane (**21**)*

2-Cyano-5-oxoheptanoic acid ethylester (15) (9.4 g, 48 mmol) in benzene (25 mL) in the presence of 4.2 mL (75 mmol) ethyleneglycol and 0.2 g toluene sulfonic acid was refluxed for 6 h. The reaction water was removed continuously by a separatory funnel. After neutralization with 2% NaOH (15 mL), the resulting solution was washed with water, then dried with anhydrous K_2CO_3. Distillation of the product yielded 9.6 g (40 mmol) **21** (83%); bp 135°C/1.0 Torr (1 Torr = 133.3 Pa). ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.92 (t, J = 7.5 Hz, 3H), 1.34 (t, J = 7.1 Hz, 3H), 1.65 (quart, J = 7.5 Hz, 2H), 1.84 (t, J = 6.7 Hz, 2H), 2.06 (m, 2H), 3.66 (t, J = 6.7 Hz, 1H), 3.97 (s, 4H), 4.30 (quart, J = 7.1 Hz, 2H). MS (T_d 130°C, 70 eV, *m/e*): 87 (15%), 101 (100), 124 (7), 154 (3), 198 (12), 212 (32), M^+ + 1 = 241 (21). Anal. calcd.: C 59.73, H. 7.94, N 5.80; found: C 59.87, H 8.07, N 5.68%.

*2-(3-Cyanopropyl)-2-ethyl-1,3-dioxolane (**22**)*

To 7.8 g (32 mmol) **21** dissolved in dimethyl sulfoxide (2.5 mL) were added 1.89 g NaCl and water (1.18 mL). The stirred mixture was heated to 180°C for 7 h. After cooling, water (80 mL) was added and the resulting solution was extracted 5 times with 40-mL portions of ether. The combined extracts were dried with anhydrous $MgSO_4$. Distillation of the product yielded 3.7 g (22 mmol, 69%) **22**; bp 95°C/0.85 Torr. ^{1}H NMR (CD_3Cl, 250 MHz) δ: 0.92 (t, J = 7.5 Hz, 3H), 1.64 (quart, J = 7.5 Hz, 2H), 1.78 (m, 4H), 2.42 (m, 2H), 3.97 (s, 4H). MS (T_d 100°C, 70 eV, *m/e*): 57 (55%), 68 (32), 96 (50), 101 (100), 110 (10), 140 (96), 154 (14), M^+ = 169 (6). Anal. calcd.: C 63.88, H 8.93, N 8.27; found: C 63.95, H 8.97, N 8.14%.

*2-(4-Aminobutyl)-2-ethyl-1,3-dioxolane (**23**)*

To a suspension of 1 g $LiAlH_4$ in 20 mL dry ether a solution of 3.12 g (18 mmol) **22** in 25 mL dry ether was introduced under stirring (1 h). After 4 h reflux ice was added. Work-up afforded 2.4 g (13.8 mmol) **23** (76%); bp 110°C/0.75 Torr. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.90 (t, J = 7.5 Hz, 3H), 1.52 (m, 4H), 1.66 (m, 4H), 2.72 (t, J = 6.8 Hz, 2H), 3.97 (s, 4H). MS (T_d 100°C, 70 eV, *m/e*): 56 (23%), 57 (26), 73 (5), 82 (10), 84 (5), 101 (100), 102 (5), 128 (12), 144 (18), M^+ = 173 (<1). Anal. calcd.: C 62.39, H 11.05, N 8.08; found: C 62.33, H 11.15, N 7.97%. The following compounds (**24**, **25**, **26**, **27**, and **2**) were prepared as described for the lower homologues **16**, **18**, **10**, and **1**.

*2-(4-[(N-Benzyloxycarbonyl-β-alanine)-aminobutyl]-2-ethyl-1,3-dioxolane **24***

Educts: 11.5 g (66 mmol) **23**, 22.7 g (66 mmol) **15**. Yield: 21 g (55 mmol) **24** (83%); mp 48–50°C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.90 (t, J = 7.5 Hz, 3H), 1.43 (m, 4H), 1.63 (m, 4H), 2.41 (t, J = 5.6 Hz, 2H), 3.27 (quart, J = 5.6 Hz, 2H), 3.52 (quart, J = 5.6 Hz, 2H), 3.93 (s, 4H), 5.12 (s, 2H), 5.54 (s, 1H), 5.62 (s, 1H), 7.38 (s, 5H). MS (T_d 130°C, 70 eV *m/e*: 57 (9%), 91 (56), 101 (100), 144 (12), 227 (4), 333 (5), 349 (15), M^+ = 378 (4). Anal. calcd.: C 63.4, H 7.99, N 7.40; found: C 63.86, H 8.41, N 7.37%.

*Propionyl-dethia(dicarba)-pantetheine **26***

Educts: 5.7 g (15 mmol) **24**, 2.2 g (17 mmol) (−)-(*R*)-pantolactone.

Yield: 2.5 g (7.5 mmol) **26** (50%); mp 57–59°C. ^{1}H NMR (D_2O, 250 MHz) δ: 0.90 (s, 3H), 0.95 (s, 3H), 1.03 (t, J = 7.5 Hz, 3H), 1.56 (m, 4H), 2.57 (t, J = 6.4 Hz, 2H), 2.66 (m, 4H), 3.27 (t, J = 6.4 Hz, 2H), 3.56 (AB, J = 12 Hz, 2H), 3.63 (m, 2H), 4.10 (s, 1H). MS (T_d 130°C, 70 eV, *m/e*: 57 (41%), 71 (100), 100 (26), 113 (26), 130 (37), 171 (16), 200 (25), 227 (12), 258 (7), 282 (3), M^+ = 330 (0.2). Anal. calcd.: C 58.16, H 9.15, N 8.48; found: C 57.66, H 9.32, N 8.51%.

*Propionyl-dethia(dicarba)-pantetheine-4′-phosphate (**27**)*

Educts: 0.9 g (2.7 mmol) **26**, 2.2 g (6.6 mmol) cyanoethylphosphate (6). Yield: 0.59 g (1.1 mmol) **27** (41%). ^{1}H NMR (D_2O, 250 MHz) δ: 0.90 (s, 3H), 0.95 (s, 3H), 1.03 (t J = 7.5 Hz, 3H), 1.56 (m, 4H), 2.57 (t, J = 7.1 Hz, 2H), 2.66 (m, 4H), 3.27 (t, J = 7.1 Hz, 2H), 3.56 (AB, J = 12 Hz, 2H), 3.63 (t, J = 7.1 Hz, 2H). ^{31}P NMR (D_2O, 121.49 MHz, H_3PO_4 ext.) δ: 5.0 (s). FAB-MS: 55 (81%), 70 (29), 113 (52), 154 (42), 229(100), 294 (56), 319 (26), 370 (39), 451 (15), 529 (10), $M^+ + 2$ = 547 (18). Anal. calcd.: C 45.51, H 6.92, N 6.63; found: C 45.24, H 7.18, N 6.54%.

*Propionyl-dethia(dicarba)-coenzyme A (**2**)*

Educts: 0.52 g (0.95 mmol) **27**, 1.194 g (2.48 mmol) **11**. Yield: 0.102 g (0.12 mmol) mixture of **2** and **2***a*. After separation on DE-32 cellulose (see **1** and ref. 14): 38 mg (0.045 mmol) **2***a* and 40 mg (0.048 mmol) **2**. ^{1}H NMR (D_2O, 400 MHz) δ: 0.74 (s, 3H), 0.86 (s, 3H), 0.97 (t, J = 7.5 Hz, 3H), 1.45 (m, 4H), 2.43 (t, J = 6.2 Hz, 2H), 2.51 (m, 4H), 3.10 (t, J = 6.0 Hz, 2H), 3.46 (t, J = 6.0 Hz, 2H), 3.66 (ABX, J_1 = 11.2 Hz, J_2 = 5.4 Hz, 2H), 4.00 (s, 1H), 4.22 (s, 2H), 4.40 (m, 1H), 4.64 (m, 1H), 6.18 (d, 1H), 8.26 (s, 1H), 8.56 (s, 1H). ^{31}P NMR (D_2O, 121.49 MHz, H_3PO_4 ext.) δ: −0.3 (s), −15.1 (d, J = 21.9 Hz), −15.6 (d, J = 21.9 Hz). FAB-MS of **2**, *m/e*: 832 (M^+ + 1) (calcd. 831.458).

S-*2-Oxobutyl-coenzyme A* ***3*** was synthesized by reacting, under argon, 25 mg (32 μmol) coenzyme A (Li_3-salt) in 2.5 mL 0.04 M Li_2CO_3 solution with 25 mg (166 μmol) 1-bromo-2-butanone dissolved in 5 mL ethanol. After 12 h the solution was added dropwise to 150 mL acetone and the precipitate collected by centrifugation. The product was purified by chromatography on DE-32 cellulose (see **1** and ref. 14). Yield: 13 mg (15 μmol, 47%). ^{1}H NMR (D_2O, 400 MHz) δ: 0.72 (s, 3H), 0.86 (s, 3H), 0.98 (t, J = 7.5 Hz, 3H), 2.42 (t, J = 6.2 Hz, 2H), 2.57 (t, J = 6.4 Hz, 2H), 2.63 (quart, J = 7.5 Hz, 2H), 3.28 (t, J = 6.2 Hz, 2H), 3.44 (t, J = 6.4 Hz, 2H), 3.65 (ABX, 2H), 3.97 (s, 1H), 4.20 (m, 2H), 4.54 (m, 2H), 6.13 (d, J = 6.2 Hz, 1H), 8.24 (s, 1H), 8.54 (s, 1H). FAB-MS *m/e*: 862 (M^+ + 1) (calcd. 861.363).

*Ezymatic carboxylation of the propionyl-coenzyme A analogues **1**, **2**, and **3** to the methylmalonyl-coenzyme A analogues **4**, **5**, and **6***

In a typical experiment 10 μmol propionyl-CoA analogue was incubated with 30 μmol oxaloacetate, 12 μmol NADH, 6 U propionyl-CoA–oxaloacetate transcarboxylase, and 5 U lactate dehydrogenase in 5 mL potassium phosphate buffer (0.05 M, pH 7.2) for 3 h at room temperature. For chromatographic purification see propionyl-CoA analogues. Yield: ca. 5 μmol (50%). ^{1}H NMR of **4** (D_2O, 250 MHz) δ: 0.73 (s, 3H), 0.88 (s, 3H), 1.21 (d, J = 7.5 Hz, 3H), 1.71 (quint, J = 6.6 Hz, 2H), 2.45 (t, J = 7.4 Hz, 2H), 2.65 (m, 2H), 3.13 (t, J = 6.6 Hz, 2H), 3.45 (t, J = 7.4 Hz, 2H), 3.55 (m, 1H), 3.66 (unresolved ABX, 2H), 4.00 (s, 1H), 4.25 (m, 2H), 6.20 (d, J = 6.2 Hz, 1H), 8.28 (s, 1H), 8.58 (s, 1H). ^{1}H NMR of **5** (D_2O, 250 MHz) δ: 0.74 (s, 3H), 0.86 (s, 3H), 1.24 (d, J = 7.6 Hz, 3H), 1.50 (m, 4H), 2.45 (t, 2H), 2.63 (m, 2H), 3.15 (t, 2H), 3.48 (t, 2H), 3.57 (m, 1H), 3.70 (ABX, unresolved, 2H), 4.02 (s, 1H), 4.25 (s, 2H), 4.58 (m, 1H), 6.20 (d, J = 6.2 Hz, 1H), 8.29 (s, 1H), 8.59 (s, 1H). ^{1}H NMR of **6** (D_2O, 250 MHz) δ: 0.74 (s, 3H), 0.87 (s, 3H), 1.28 (d, J = 7.5 Hz, 3H), 2.49 (t, J = 6.2 Hz, 2H), 2.64 (t, J = 6.4 Hz, 2H), 3.34 (t, J = 6.2 Hz, 2H), 3.48 (t, J = 6.4 Hz, 2H), 3.67 (unresolved ABX, 2H), 3.78 (quart, 1H), 4.01 (s, 1H), 4.25 (m, 2H), 4.60 (m, 1H), 6.22 (d, J = 6.2 Hz, 1H), 8.33 (s, 1H), 8.58 (s, 1H).

*Enzymatic carboxylation and isomerization of the propionyl-coenzyme A analogues **1**, **2**, and **3** to the succinyl-coenzyme A analogues **7**, **8**, and **9***

In a typical experiment 4 μmol propionyl-CoA analogue was incubated with 16 μmol oxaloacetate, 20 μg coenzyme B_{12}, and a mixture of propionyl-CoA–oxaloacetate transcarboxylase, methylmalonyl-CoA epimerase, and methylmalonyl-CoA mutase, 1 U of each, in 2 mL of potassium phosphate buffer (0.2 M, pH 6.8) for 12 h at room temperature. The reaction was terminated at 0°C with 0.1 M perchloric acid (→ pH 3). After removal of the precipitated proteins by centrifugation the pH of the solution was adjusted to 6 with 2 M K_2CO_3. Removal of the crystalline potassium perchlorate was followed by lyophilization. Yield: ca. 40%. ^{1}H NMR of **7** (D_2O, 250 MHz) δ: 0.55 (s, 3H), 0.72 (s, 3H), 1.50 (quint, J = 5.8 Hz, 2H), 2.25 (m, 3H), 2.40 (t, J = 5.8 Hz, 2H), 2.53 (d, J = 6.2 Hz, 2H), 2.95 (t, J = 5.8 Hz, 2H), 3.30 (t, J = 6.8 Hz, 2H), 3.53 (ABX, J = 12 Hz, J_{HP} = 6 Hz, 2H), 3.86 (s, 1H), 4.10 (m, 2H), 6.06 (d, J = 7.5 Hz, 1H), 8.15 (s, 1H), 8.43 (s, 1H). ^{1}H NMR of **8** (D_2O, 400 MHz) δ: 0.75 (s, 3H), 0.86 (s, 3H), 1.48 (m, 4H), 2.46 (m, 4H), 2.75 (t, 2H), 3.08 (t, 2H), 3.44 (t, 2H), 3.65 (unresolved ABX, 2H), 3.97 (s, 1H), 4.20 (m, 2H), 4.54 (m, 1H), 6.15 (d, J = 6.2 Hz, 1H), 8.24 (s, 1H), 8.54 (s, 1H). ^{1}H NMR of **9** (D_2O, 400 MHz) δ: 0.72 (s, 3H), 0.86 (s, 3H), 2.46 (m, 4H), 2.58 (t, J = 6.4 Hz, 2H), 2.84 (t, J = 7.2 Hz, 2H), 3.30 (t, J = 6.2 Hz, 2H), 3.46 (t, J = 6.4 Hz, 2H), 3.65 (unresolved ABX, 2H), 3.97 (s, 1H), 4.20 (m, 1H), 6.13 (d, J = 6.2 Hz, 1H), 8.24 (s, 1H), 8.54 (s, 1H).

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