SYNTHESIS OF β-OXO THIOL ESTERS RELATED TO BILE ACID BIOSYNTHESIS

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A postulated intermediate of bile acid biosynthesis, 24-oxo-3α,7α,12α-trihydroxy-5β-cholestan-26-oyl CoA was chemically synthesized from cholic acid.

KEY WORDS β-oxo thiol ester; bile acid; N-acetylcysteamine; coenzyme A; 24-oxo-3α,7α,12α-trihydroxy-5β-cholestan-26-oyl CoA; cholyl-CoA

Side chain degradation of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) in liver proceeds by a mechanism similar to β-oxidation of fatty acids, giving the primary bile acid cholic acid (**5**) (Chart 1).[1] In this transformation, the intermediary roles of THCA-CoA (**1**), Δ^{24}-THCA-CoA (**2**) and 24-hydroxy-THCA-CoA (**3**) seem to be established, except for 24-oxo-THCA-CoA (**4**). We have recently found the ethyl ketone (**6**) in the saponified product obtained from incubation of Δ^{24}-THCA with rat liver homogenate, and postulated that this ketone (**6**) is derived from a genuine incubation product 24-oxo-THCA-CoA (**4**) by alkaline hydrolysis followed by decarboxylation.[2] To verify this hypothesis and definitely identify **4** as an obligatory intermediate of bile acid biosynthesis, an authentic specimen of **4** is essential. In this paper we describe chemical synthesis of **4**, together with its model compounds ethanethiol ester (**9**) and *N*-acetylcysteamine (NAC) ester (**1 4**).

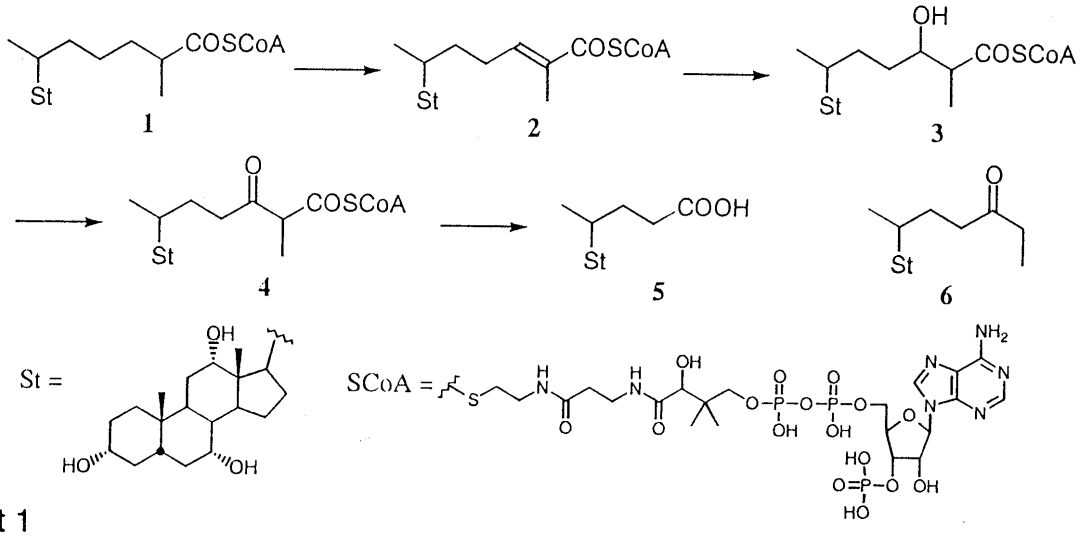

Chart 1

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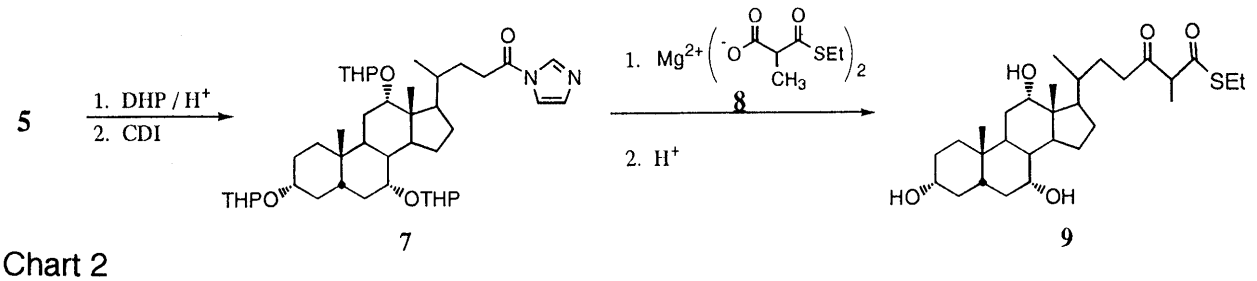

Chart 2

Since β-oxo carboxylic acid is extremely labile to decarboxylation,[2] it seems difficult to prepare the desired thiol esters by thioesterification of the corresponding β-oxo acid. An alternative method for preparation of β-oxo esters would be decarboxylative acylation of half thiol ester of malonic acid (Chart 2).[3] In this context, tris-tetrahydropyranyl (THP) ether of cholic acid was treated with 1,1'-carbonlyldiimidazole to give the imidazolide (**7**), which, without isolation, was treated with magnesium salt (**8**) of 2-methylmalonic acid ethanethiol half ester.[4] The condensation proceeded well, and ethanethiol ester of 24-oxo-THCA (**9**) was obtained, after acidic deprotection of THP group, in 71% yield. However, this procedure was found to be inapplicable to the NAC (and hence CoASH) half ester, probably because of inappropriate formation of magnesium salt due to the presence of amide moiety.

Then, we turned our attention to ester interchange reaction of thioglycolate (Chart 3).[5] The effectiveness of this method was first demonstrated by preparation of cholyl-CoA as follows. The imidazolide (**7**) described above was treated with thioglycolic acid in THF to give the thioglycolate (90%), which , after removal of THP group, was then exposed to 2 equivalents of CoASH in THF-H_2O (2 : 1) at pH 8 (by addition with 1N NaOH) at ambient temperature for 30 min, to give cholyl-CoA[6] in 40% overall yield. According to this strategy, the synthetic precursor of the final target **4** should be the 24-oxo thioglycolate (**13**), and this has to be prepared by bypassing the labile 24-oxo-26-oic acid. The 24-oxo ethyl ester (**10**) prepared from **7** in the same manner as described for the ethanethiol ester (**9**), was treated with ethyleneglycol at reflux in benzene containing *p*-toluenesulfonic acid and then saponified with 5% KOH-methanol to afford the ethyleneacetal (**11**, 73%). This acid was condensed with methyl thioglycolate by action of bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOPCl)[7] in THF containing triethylamine at reflux to afford the thiolester (**12**) in 87% yield. Refluxing of **12** in THF-6N HCl (4 : 1) for 2 hr effected hydrolysis of both ethyleneacetal and methyl ester groups, leaving the thiolester bond intact, to give the acid (**13**). Coupling of **13** with NAC (1 eq) in THF-H_2O (2 : 1) at pH 8 at ambient temperature for 15 min gave the NAC ester (**14**) in 37% yield from **12**. Finally, similar ester interchange reaction of **13** with CoASH (2 eq) afforded 24-oxo-THCA-CoA (**4**)[8] in 57% yield. ^{13}C-NMR spectra of the synthetic **4**, **9** and **14** have pairs of signals due to the diastereotopic C-25.

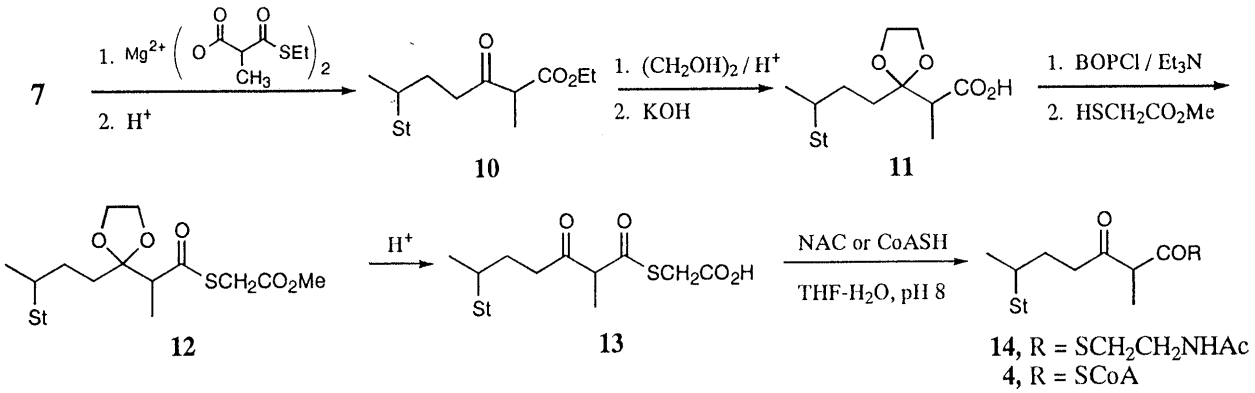

Chart 3

In summary, the present investigation completed synthesis of the long-sought 24-oxo-THCA-CoA (**4**), which should provide a valuable tool for study of the mechanism of the final step of bile acid biosynthesis.

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8) ^{1}H-NMR (d_6-DMSO): 0.55 (s, 18-H_3), 0.73 and 0.91 (two s, gem-diMe), 0.78 (s, 19-H_3), 0.86 (d, J=5.6 Hz, 21-H_3), 1.19 (m, 27-H_3), 2.91 (m, SCH_2), 3.59 (m, 3-H), 3.75 (12-H), 3.85 (m, 12-H), 4.00 (q, J=7.0 Hz, 25-H), 4.16, 4.39, 4.71 and 4.80 (5H, ribose-H), 5.97 (d, J=4.9 Hz, anomeric H), 7.74 and 8.19 (two NH), 8.42 and 8.63 (two adenine H). ^{13}C-NMR (d_6-DMSO): 13.29 and 13.33 (C-27), 17.04 and 17.11 (C-21), , 196.44 (C-26), 60.43 and 60.48 (C-25), 205.11 and 205.28 (C-24). FABMS: $[M - H]^-$ m/z 1212. Treatment of **4** with NaOH-EtOH afforded, as expected, quantitatively the ethylketone **6**.[2] Preliminary incubation experiment of **4** with rat liver homogenate (10,000 x g precipitate, in the *absence* of ATP and CoA) indicated its efficient conversion into cholic acid (**5**).

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