Microbial Transformation of Dehydrocholic Acid into Steroid Dimers containing the Benzene Ring

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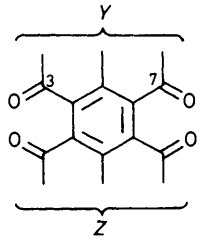
Dehydrocholic acid has been converted by *Streptomyces gelaticus* into steroid dimers (4,4' : 6,6'- or 4,6' : 4',6-bisteroids) in which carbon atoms 4, 4', 5, 5', 6, and 6' comprise a benzene nucleus.

We have found that *Streptomyces gelaticus* is able to convert dehydrocholic acid into an unknown acidic product which shows strong u.v. absorptions at 229, 285, and 333 nm.[1] No reports of products of the microbial transformation of bile acids[2] and other steroids[3] showing such u.v. absorptions have been published hitherto.

The product was isolated as described previously[1] and esterified with CH_2N_2. Preparative t.l.c. on silica gel in benzene/EtOAc/AcOH (70:25:5) afforded three components. Analytical and mass spectrometric data for the three esters, which all had m.p. >330 °C, were consistent with the compositions $C_{50}H_{62}O_{10}$, $C_{48}H_{58}O_{10}$, and $C_{46}H_{54}O_{10}$, respectively. The three u.v. spectra were almost identical, and resembled that of acetophenone [230 (log ε 4.5), 285 (4.0), and 334 nm (3.7)]. Each of the i.r. spectra showed two C=O bands near 1710 and 1740 cm^{-1}. The ^1H n.m.r. spectra of the C_{50} and the C_{46} esters exhibited signals at δ 0.87 (6H, d, *J* 7 Hz), 1.15 (6H, s), 1.36 (6H, s), and 3.67 (6H, s), and δ 1.12 (6H, s), 1.19 (6H, d), 1.36 (6H, s), and 3.68 (6H, s), respectively. Superposition gave a spectrum similar to that of the C_{48} ester, indicating that each ester has two 18-Me, two 19-Me, and two CO_2Me. Besides these products, *S. gelaticus* has also produced 7α-hydroxy-3,12-dioxochol-4-en-24-oic acid and the 23,24-dinor analogue.[1] This indicates the possibility that the C_{24} and C_{22} acids or related compounds self- and cross-dimerize to produce the corresponding C_{48}, C_{46}, and C_{44} dicarboxylic acids. It is therefore presumed from the spectral and the biochemical evidence that the C_{48} acid is either 3,3',7,7',12,12'-hexaoxobenzo[1,2,3-*de*:6,5,4-*d'e'*]dichol-4-en-24-oic or 3,3',7,7',12,12'-hexaoxobenzo[1,2,3-*de*:4,5,6-*d'e'*]dichol-4-en-24-oic acid (tentatively referred to here as *cis*- and *trans*-forms, respectively). The ^{13}C n.m.r. signals of the C_{46} ester [δ 136.77 and 137.71 (C-4, -4',-6, and -6'), 150.98 (C-5 and -5'), 176.63 (C-22 and -22'), 198.21 and 198.34 (C-3, -3', -7, and -7'), and 210.52 (C-12 and -12')] furnished further support for this symmetrical structure. Thus the structures of the respective C_{48}, C_{46}, and C_{44} acids may be depicted as partial formulae (**1**), (**2**), and (**3**), but their orientations are still uncertain.

To obtain further support for these structures, the partial synthesis of a *cis*-dimer has been undertaken which has the structure of rings *A*, *A'*, *B*, and *B'* of (**1**). Since phenols are dimerized oxidatively by alkaline $K_3Fe(CN)_6$,[4] we expected that cholest-5-ene-3,7-dione, which exists mainly as the monoenolic form (3-hydroxy-3,5-dien-7-one),[5] might be



(**1**) *Y* = *Z* = $C_{19}H_{29}O_3$

(**2**) *Y* = $C_{19}H_{29}O_3$, *Z* = $C_{17}H_{25}O_3$

(**3**) *Y* = *Z* = $C_{17}H_{25}O_3$

(**4**) *Y* = *Z* = $C_{22}H_{39}$

dimerized by this reagent to yield 4,4'-bisteroids which probably exist as an equilibrium mixture of 3,3'-dihydroxy-3,3',5,5'-tetraene-7,7'-dione and 7,7'-dihydroxy-4,4',6,6'-tetraene-3,3'-dione. Also it might be expected that the latter bisteroid would be further oxidized in a similar manner to a 4,4' : 6,6'-bisteroid similar to the microbial products. Oxidation of the dione according to the method of Sarkanen and Wallis[6] gave two dimerization products with m.p.s 249—250 and 283—284.5 °C, each in *ca.* 10% yield. As expected, data for both products were consistent with the composition $C_{54}H_{78}O_4$; they exhibited u.v. absorptions at 228, 286, and 335 nm and 230, 285, and 332 nm, respectively. The former showed ^{13}C n.m.r. signals at δ 136.50 and 138.17 (C-4, -4', -6, and -6'), 152.17 (C-5 and -5'), and 199.17 and 201.17 (C-3, -3', -7, and -7') and the latter at δ 137.17 and 137.50 (C-4, -4', -6, and -6'), 152.00 (C-5 and -5'), and 199.50 and 201.84 (C-3, -3', -7, and -7'). Although identification of the *cis*- and *trans*-forms is uncertain, the partial formula common to these cholesterol dimers is (**4**). The i.r. and ^1H n.m.r. spectra supported these structures. The formation of these two products is explained on the basis of initial 4,4'-, 6,6'-, 4,6'-, or 4',6-coupling, since the 3-hydroxy-3,5-dien-7-one is probably in equilibrium with another enol, the 7-hydroxy-4,6-dien-3-one, in alkaline solution. The results offer chemical corroboration for structures (**1**)—(**3**), and also seem to provide a new chemical method of dimerizing a 2-ene-1,5-dione to a benzene ring, although a variety of reactions for the formation of the benzene nucleus have been reported.[7] However, further study is desirable as to whether this method is applicable to acyclic compounds as well as to rigid systems such as steroids.

In contrast to the chemical dimerization, in which both *cis*- and *trans*-products were formed, incubation of cholest-5-ene-3,7-dione with *S. gelaticus* yielded only the product with m.p. 249—250 °C. In the transformation of dehydrocholic acid with this organism, of a possible six isomers only three dimers, the C_{48}, C_{46}, and C_{44} acids, were formed, and the C_{48} acid was also produced from this bile acid by *Arthrobacter simplex*,[2] capable of dehydrogenating 3-oxo bile acids to 3-oxo-4-ene bile acids. The formation of single isomers in these microbial transformations indicates that the microbial coupling is stereoselective. From the results of these biotransformations and the mechanism proposed for the formation of the cholesterol dimers, it seems likely that the immediate precursors of (**1**) and (**3**) in the microbial dimerization are 3,7,12-trioxochol-4-en-24-oic acid and its 23,24-dinor analogue, respectively, and that cross-coupling of these acids yields (**2**). To our knowledge, no instance of this type of microbial dimerization of steroids[2,3] or other compounds[8] containing a 2-ene-1,5-dione structure has been noted previously.

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