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Naturally Occurring Lactones and Lactames

II. The Absolute Configuration of γ-Methyltetronic Acid, Carolinic Acid, and Carolic Acid

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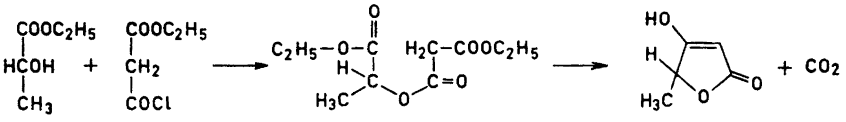
The *Penicillium charlesii* G. Smith metabolites (−)-γ-methyltetronic acid (*6*), (+)-carolinic acid (*7*), and (+)-carolic acid (*9*) have all been shown to possess the *R*-configuration. (*S*)-(+)-γ-Methyltetronic acid was synthesized from ethyl (*S*)-lactate by two different methods and thus shown to be the stereoisomer of the naturally occurring γ-methyltetronic acid. The two other metabolites have earlier been degraded to (+)-α-bromo-γ-methyltetronic acid, which now is found to be enantiomeric to (*S*)-(−)-α-bromo-γ-methyltetronic acid synthesized from ethyl (*S*)-lactate.

The tetronic acid structure not only appears in the vitamin, L-ascorbic acid, but in addition is frequently found in natural products. In examining the metabolites of *Penicillium charlesii* G. Smith and some other fungi, Raistrick and his collaborators [1–3] discovered a series of compounds all derived from tetronic acid and they were designated carolinic, carolic, carlic, *etc.* acids (for structures *cf.* (*6*), (*7*), (*8*), (*9*)) after the name of the fungus.

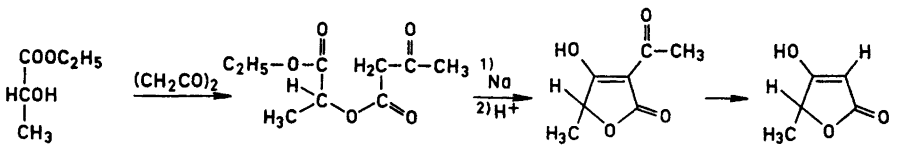
As a help in tracing the biosynthetic pathways of these acids it would be useful to know their absolute configuration. It seemed reasonable to attempt synthesis and as a suitable compound the simplest member of this group, (−)-γ-methyltetronic acid, was chosen. As the racemate, γ-methyltetronic acid has been known since 1911, when it was synthesized by Benary.[4] Since then many alkyltetronic acids have been prepared by different methods, but all have been optically inactive.

Two methods used for the synthesis of racemic γ-methyltetronic acid seemed promising for preparing optically active forms and were tried:

a. Synthesis after Haynes and Stanners,[5] which involves reaction of ethyl lactate with ethoxycarbonylacetyl chloride to give ethyl α-(ethoxycarbonylacetoxy)-propionate and cyclization and decarboxylation of the product to give γ-methyltetronic acid:

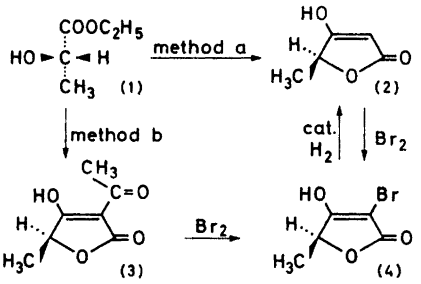


b. Synthesis after Lacey [6] involving the interaction of ethyl lactate with diketene to give ethyl α-(acetoacetoxy)-propionate and cyclization of the product to α-acetyl-γ-methyltetronic acid which then was hydrolyzed to γ-methyltetronic acid:



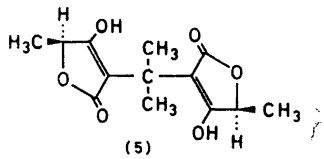
Using method a it was possible with ethyl (*S*)-lactate (*1*) to obtain an optically active γ-methyltetronic acid (*2*) [m.p. 109—111°, $[\alpha]_{546}$ +17.5°], for which the absolute configuration must be identical to that of (*S*)-lactic acid.

Following method b it was possible to prepare (*S*)-α-acetyl-γ-methyltetronic acid (*3*), but the hydrolysis to (*S*)-(+)-γ-methyltetronic acid (*2*) could not be



achieved in a satisfactory manner, neither in acidic nor in alkaline medium. On the other hand, Raistrick and collaborators [1] have found that (+)-carolinic acid (*7*) and (+)-carolic acid (*8*) suffer extrusion of the acyl side chain by bromine to yield (+)-α-bromo-γ-methyltetronic acid. This was tried with (*S*)-α-acetyl-γ-methyltetronic acid (*3*), which yielded (*S*)-(—)-α-bromo-γ-methyltetronic acid (*4*) (m.p. 164—166°, $[\alpha]_{546}$ —5.03), reducible catalytically to (*S*)-(+)-γ-methyltetronic acid (*2*). It is also possible to revert the last reaction, since treatment of (*2*), with bromine yields (*4*).

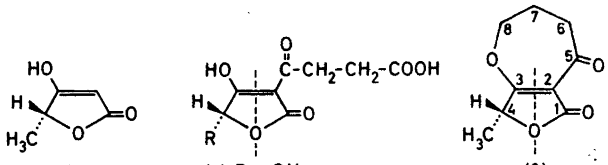
As a further check on the assignment of configuration to (*S*)-γ-methyltetronic acid (*2*) the compound was reacted with acetone to give the dextrorotatory isopropylidene derivative (*5*). The naturally occurring (—)-γ-methyltetronic acid yielded with acetone the (—)-stereoisomer.[1]



(5)

The conclusion of the above work is then that the naturally occurring (—)-γ-methyltetronic acid (*6*), (+)-carolinic acid (*7*), and (+)-carolic acid (*9*) all have the *R*-configuration.

The mold tetronic acids have a unique structural relationship to L-ascorbic acid as well as to fatty acids and appear to be condensation products of two simple molecules. By labeled acetate studies on carlosic acid (*8*) and carolic acid (*9*) Lybing and Reio [7] have clearly demonstrated the regularly alterna-



(6) (7): R = —CH_3 (8): R = —CH_2COOH (9)

tively high and low radioactivity in the carbon atoms of the α-side chain. This indicates that the side chain is formed from acetate units. Evidence that this polyacetic acid condensation actually involves malonate is reported by Bentley and coworkers,[8,9] who found that in cultures of *P. charlesii* labeled acetic acid furnished carbons 1, 2, 5, 6, 7, and 8 of carolic acid (*9*), whereas labeled malonic acid furnished only carbons 1, 2, 5, and 6. These carbons are, therefore, believed to be derived from condensation of one acetyl coenzyme A and two malonyl coenzyme A units. Further, Lybing and Reio [7] as well as the Bentley group [8,9] propose that the carbons in which neither acetate nor malonate is incorporated (carbons 3, 4, 9, and 10 in carlosic acid (*8*), 3, 4, and 9 in carolic acid (*9*)) can be derived from a C_4 dicarboxylic acid, succinic acid or malic acid. Although this proposal seems rather unlikely, since labeling of carbons 3, 4, and 9 in carolic acid (*9*) would have been expected from the operation of the citric acid cycle, the present assignment of configuration to some of the mold tetronic acids is in agreement with the proposal of malic acid as a precursor, since it will require the incorporation of the natural (*R*)-malic acid.

EXPERIMENTAL

The 60 Mc/s spectra were recorded on a Varian A 60. All spectra were obtained from approx. 20 % solutions in dimethyl sulfoxide-d_6 with tetramethylsilane as standard. All values are δ[ppm] relative to TMS = 0.

Ethyl (S)-*lactate*. The ester (C.H. Boehringer Sohn, Ingelheim am Rhein) had $[\alpha]_D^{25}$ −10.1 (liq., l=0.2), n_D^{25} 1.4130, d_4^{15} 1.0315.

Ethoxycarbonylacetyl chloride. Potassium ethyl malonate (200 g) prepared from diethyl malonate [12] was reacted with 140 g of thionyl chloride to give 65 g (46 %) of ethoxycarbonylacetyl chloride, b.p. 72–75°/15 mm (Lit.[13] 75–77°/17 mm).

Ethyl (S)-α-(*ethoxycarbonylacetoxy*)-*propionate.* Prepared according to Haynes and Stanners [5] from 94 g of ethyl (*S*)-lactate, 150 ml of pyridine, and 68.5 g of ethoxycarbonylacetyl chloride to give 20.8 g (20 %) of ethyl (*S*)-α-(ethoxycarbonylacetoxy)-propionate, b.p. 97–100°/0.1 mm, $[\alpha]_D^{25}$ −37.9° (liq., l=0.1). (Found: C 51.83; H 7.00. Calc. for $C_{10}H_{16}O_6$: C 51.72; H 6.94).

(S)-γ-*Methyltetronic acid* (2). a. Cyclization [6] of 20.0 g of ethyl- (*S*)-α-(ethoxycarbonylacetoxy)-propionate with sodium gave 0.167 g (1.7 %) of (*S*)-methyltetronic acid. Repeated crystallizations from ethyl acetate-petrol ether raised the m.p. from 101–106° to 109–110°, $[\alpha]_D^{25}$ +14.7°, $[\alpha]_{546}^{25}$ +15.0° (c=0.193, H_2O). (Found: C 52.14; H 5.30. Calc. for $C_5H_6O_3$: C 52.63; H 5.30). NMR: *ca.* 3–6 (OH), 2.38 (C-4, H, quartet, J=3.3 cps), 2.42 (C-2, H, singlet) 0.69 (C-5, H_3, doublet, J=3.3 cps). (Lit. for stereoisomer:[1] m.p. 115°, $[\alpha]_{546}$ −21°).

b. (*S*)-α-Bromo-γ-methyltetronic acid (*4*) (40 mg) was catalytically reduced in 10 ml of 50 % aqueous acetic acid using 10 % palladium on charcoal. In 15 min the reduction was complete and corresponded to an uptake of 1 mol of hydrogen. The catalyst was filtered off and washed well with water. The filtrate and washings were extracted five times with an equal volume of ether, the ethereal extracts dried over anhydrous magnesium sulfate and the ether removed *in vacuo*. A crystalline product (22 mg) remained which after recrystallization from ethyl acetate-petrol ether melted at 109–111°, $[\alpha]_D^{25}$ +17.1°, $[\alpha]_{546}^{25}$ +17.5° (c=0.189, H_2O). IR spectrum in KBr identical to that made of compound under a.

Ethyl (S)-α-(*acetoacetoxy*)-*propionate.* This was prepared according to Lacey [6] from 59 g (0.5 mol) of ethyl (*S*)-lactate and 0.5 ml of triethylamine, to which is added during an hour 42 g (0.5 mol) of diketene. The temperature was maintained at 65–75° during the reaction as well as 30 min after. Yield: 71.0 g (70 %) of ethyl (*S*)-α-(acetoacetoxy)-propionate, b.p. 88–90°/0.3 mm, $[\alpha]_D^{25}$ −36.1° (liq., l=0.1) (Found: C 53.60; H 7.07. Calc. for $C_9H_{14}O_5$: C 53.46; H 6.98).

(S)-α-*Acetyl*-γ-*methyltetronic acid* (3). Cyclization [6] of 70.0 g of ethyl (*S*)-α-(acetoacetoxy)-propionate with sodium gave after distillation, solidification, and recrystallization from ether-petrol ether 20.2 g (35 %) of yellow crystals, m.p. 63–66°, $[\alpha]_D^{25}$ −58.3° (c=0.205, H_2O). (Found: C 53.62; H 5.76. Calc. for $C_7H_8O_4$: C 53.84; H 5.16). NMR: 4.50 (OH, sharp), 2.39 (C-4, H, quartet, J=3.3 cps), 1.20 (C-2, $OCCH_3$, singlet), 0.70 (C-5, H_3, doublet, J=3.3 cps).

(S)-α-*Bromo*-γ-*methyltetronic acid* (4). a. To a suspension of 116 mg (1.02 mmol) (*S*)-γ-methyltetronic acid in chloroform was added 0.159 g (1 mmol) of bromine in chloroform. After 10 min of standing the solution was evaporated *in vacuo* to yield a crystalline product, m.p. 148–150°. Recrystallized from benzene it melted at 158–160°; yield 53.9 %. $[\alpha]_{546}^{25}$ −3.71 (c=0.296, H_2O). (Found: C 30.81; H 2.53; Br 40.70. Calc. for $C_5H_5O_3Br$: C 31.10; H 2.61; Br 41.40). NMR: 3.88 (OH, broad), 2.46 (C-4, H, quartet, J=3.5 cps), 0.70 (C-5, H_3, doublet, J=3.5 cps). (Lit. for stereoisomer:[1] m.p. 172°, $[\alpha]_{546}$ +9.47°).

b. (*S*)-α-Acetyl-γ-methyltetronic acid (*3*) (100 mg) was dissolved in 2 ml of 50 % aqueous acetic acid and a standardized solution of bromine in the same solvent added and a sharp end-point was obtained after the equivalent of 2 mol of bromine had been added. The solution was lyophilized over solid KOH to give 70 mg of a crystalline mass. After several recrystallizations from benzene the product (8 mg) melted at 164–166° and on mixing with the compound prepared under a. no depression of melting point was noticed, $[\alpha]_{546}^{25}$ −5.03° (c=0.141, H_2O).

Isopropylidene derivative of (S)-γ-*methyltetronic acid* (5). Prepared according to Ref. 1. (*S*)-γ-methyltetronic acid (249 mg) gave 230 mg of isopropylidene derivative (39.2 %). M.p. 163–168°, $[\alpha]_{546}^{25}$ +32.3° (c=0.495, H_2O) (Found: C 58.40; H 6.22. Calc. for $C_{13}H_{16}O_6$: C 58.19; H 6.02) (Lit. for stereoisomer:[1] m.p. 172°, $[\alpha]_{546}$ −39°).

Hydrolysis of (S)-α-*acetyl*-γ-*methyltetronic acid* (3). To 5 g of (*3*) was added a solution of 3 g of sodium hydroxide in 5 ml of water. After standing for 1 h at room temperature, the solution was kept at 50° for 3 h. The solution was acidified with 4 N hydrochloric acid and extracted with 3×10 ml of ether. The ethereal extracts were dried over sodium sulfate and evaporated to yield a brown oil, which could not be induced to crystallize.

b. To 1.0 g of (*3*) was added 150 ml of 0.1 N sulfuric acid and the solution was refluxed for 4 h in an atmosphere of nitrogen. The solution was extracted with 3×50 ml of ether, the ethereal extracts dried over sodium sulfate and evaporated *in vacuo* to yield a yellow oil, which crystallized on cooling. Yield 13 mg (1.7 %), m.p. 104—109°. The IR spectrum indicated impure γ-methyltetronic acid.

c. Repetition of a. with five times the amounts of starting materials did not result in any crystalline product.

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