digest of the nucleic acid is treated with periodate and then passed through a column of the cellulose at 4° in the presence of 4 *M* sodium chloride. Under these conditions the unoxidized internal fragments (containing phosphate groups at their 3′ ends) are eluted while the oxidized terminal fragment is retained. Recovery is effected by the subsequent displacement of the chloride ions by bicarbonate ions followed by elution of the column at 45° with a solution of a primary amine (in the form of its volatile bicarbonate). The conditions used for the recovery are similar to the reaction conditions used by Neu and Heppel[3] for the removal of the terminal base from a periodate-oxidized oligonucleotide by a β-elimination reaction, and thus the recovered terminal fragment is expected to be found lacking its terminal nucleoside.

Bacteriophage f2 RNA (150 mg, 84 mμmoles) was dissolved in water (5 ml), and ribonuclease T_1 (1.5 mg) was added. The pH of the reaction was kept at 7.5 by the controlled addition of 0.1 *M* NaOH. After 24 hr at 25°, 0.1 *M* sodium periodate (2.5 ml) was added. The mixture was allowed to stand for 0.5 hr at 25° and the excess periodate was then destroyed by the addition of sucrose (83 mg). After 40 min at 25° the mixture was cooled to 0°, sodium chloride (1.75 g) was added, and the solution was adjusted to pH 8.5. Aminoethylcellulose (Cellex AE, 0.4 mequiv/g) was washed with 0.5 *M* *n*-propylamine bicarbonate at 45° and then with 4 *M* NaCl (pH 8.5). The cellulose was then packed at 4° into a water-jacketed column (1 × 84 cm) and washed with the NaCl solution. The oxidized enzymic digest of the RNA was applied to the column and eluted at 4° with the 4 *M* NaCl (pH 8.5) at 8–10 ml/hr. After all the internal oligonucleotides were eluted, sufficient 0.5 *M* $KHCO_3$ (pH 8.5) was passed through at 100 ml/hr, 4°, to displace all chloride ions from the column. The potassium bicarbonate was removed by elution with water at the same temperature and flow rate. The temperature of the column was then raised to 45°, and elution of the terminal fragment was carried out with 0.5 *M* *n*-propylamine bicarbonate (pH 7.5) at 5–6 ml/hr, 45°. In model experiments where *ca.* 3 ODU (260 mμ) of uridylyl-(3′–5′)-adenosine or guanylyl-(3′–5′)-adenosine was used in place of the RNA, uridine 3′-phosphate and guanosine 3′-phosphate were obtained in 60% yield by paper chromatography of the respective propylamine bicarbonate eluates.

The entire propylamine bicarbonate eluate (200 ml) was evaporated to dryness *in vacuo*, and the volatile salt was completely removed by repeated solution of the product in water and evaporation to dryness. The product was applied to a DEAE-Sephadex A-25 column (0.6 × 20 cm). Elution was effected with 600 ml of 0.05 *M* Tris acetate buffer–7 *M* urea (pH meter reading: 8.0) containing a linear gradient of sodium acetate (0.15–0.95 *M*) at a flow rate of 2.5 ml/hr. The terminal fragment appeared as a peak (4.6 ODU at 260 mμ, 60%) at a position which corresponded to the position of elution of the octanucleotides. The position of elution of the latter was determined by the chromatography of a ribonuclease T_1 digest of f2 RNA on an identical column under the same conditions. For analysis, the peak fractions were combined, dialyzed against water, and concentrated to 1.5 ml. The product was treated with alkaline phosphatase (0.1 mg) for 20 hr at 37°. The reaction mixture was adjusted to 0.3 *M* potassium hydroxide by addition of 1 *M* KOH and was kept at 37° for 20 hr. Control experiments have shown that alkaline phosphatase does not survive this treatment nor does it have enzyme activity during the alkaline hydrolysis. Thus, there was no problem arising from the dephosphorylation of nucleotides formed by the alkaline hydrolysis. Analysis of the hydrolysate by a modification of a method previously described[4] showed cytidine as the only nucleoside present. Another preparation of the terminal fragment was directly treated with 0.3 *M* potassium hydroxide for 20 hr at 37°. The mixture was neutralized with perchloric acid, and the nucleotides present were determined by a modification of the method described by Cohn and Volkin;[5] found: Up, 93 mμmoles; Ap, 96 mμmoles; Cp, 189 mμmoles; ratio Up:Ap:Cp = 2.00:2.06:4.06.

It thus appears that the terminal fragment isolated from f2 RNA has a terminal cytidine and has the composition: (3Cp,2Up,2Ap)-Cp. Other experiments in this laboratory have shown conclusively that the terminal base of f2 RNA is adenosine[4] and that the penultimate base is cytidine. The treatment of f2 RNA with the uridine blocking agent, 1-cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide metho-*p*-toluenesulfonate, and then pancreatic ribonuclease under previously described conditions[6] yields the same amount of adenosine end group as is obtained without prior treatment with the blocking agent. Also, treating the f2 RNA with periodate under published conditions[3,7] followed by removing the terminal phosphate so produced gave a molecule which, on treating with alkali, yielded cytidine as its terminal nucleoside. These results are consistent with the isolation, after periodate oxidation, of a fragment containing a terminal cytidine as described above. Thus, together with the fact that the above fragment was produced by an enzyme that is specific for the cleavage of guanosine 3′-phosphoryl bonds, the present experiments indicate that the terminal nucleotide sequence of the f2 RNA is -Gp-(3Cp,2Up,2Ap)-CpA.

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Biogentic-Type Synthesis of an Acylphloroglucinol[1]

Sir:

Birch has postulated that polyketo acids (or esters) are formed as intermediates in the biosynthesis of phenolic compounds from acetate.[2] He concluded

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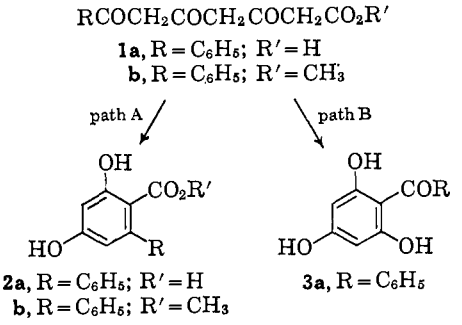
that the polyketo acids underwent carbocyclic ring closure by two routes, as shown below. Intramolecular aldol condensations (path A) would give rise to β-resorcylic acid derivatives **2**, whereas intramolecular Claisen condensations (path B) would afford acylphloroglucinols **3**.

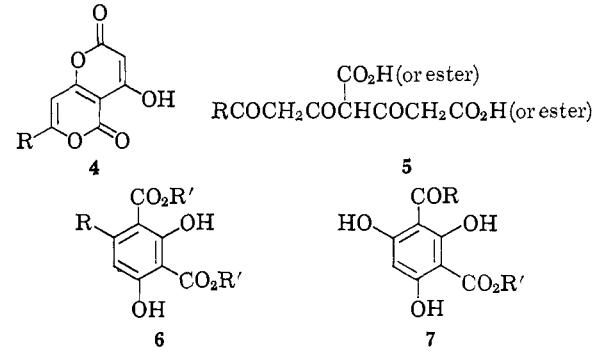


We have recently reported a number of examples of the first type (path A) of cyclization.[3] The reactions were effected in high yields with triketo acids **1** (R′ = H) in aqueous solution at pH 5.0. No formation of acylphloroglucinols **3** was observed in these reactions.

We now wish to report a Claisen-type cyclization (path B) which was undergone by the methyl ester **1b** of 7-phenyl-3,5,7-trioxoheptanoic acid. Careful treatment of acid **1a**[4] with diazomethane gave the methyl ester,[5] mp 73–76°, in good yield. Care was taken to avoid excess diazomethane since further methylation of **1b** occurred and the products were not readily separated from **1b**. Ester **1b** was treated with aqueous 2 *M* potassium hydroxide at −5° for 19 hr. Thin layer chromatography of the product mixture showed the presence of phloroglucinol **3a** and resorcylic derivatives **2a,b**, as well as trace amounts of 5-phenylresorcinol. Nmr analysis indicated that the mixture contained about 66% of phloroglucinol **3a** and 34% of resorcylic derivatives **2a,b**. 5-Phenylresorcinol was not present in measurable quantity. Benzoylphloroglucinol (**3a**) was isolated in 47% yield by chromatography on silicic acid (ether–hexane eluent) followed by recrystallization from water and from chloroform to give mp 165–166° (lit.[6] mp 165°). The structure was confirmed by a mixture melting point with authentic **3a**.[7] Cotoin, which is obtained from coto bark and other sources, is the 4-methoxy analog of **3a**.[8]

The formation of phloroglucinol **3a** was highly sensitive to experimental conditions. When methanolic 1.4 *M* potassium methoxide or aqueous potassium phosphate buffer, pH 8.5, was employed, resorcylic ester **2b**,[5] mp 120–121°, was produced in high yields. Phloroglucinol **3a** was not detected. No significant effect of magnesium ion was observed. An aqueous mixture of magnesium hydroxide and potassium hydroxide produced a mixture of **3a** and **2a,b** similar to that formed by potassium hydroxide alone. Aqueous magnesium hydroxide, pH 9.7, methanolic magnesium methoxide, and aqueous magnesium Tris buffer, pH 8.5, all gave heavily precipitated mixtures with **1b**. Ester **2b**, but none of phloroglucinol **3a**, was obtained from all three. The last two sets of conditions gave significantly slower reactions than did the others. A rationale cannot yet be proposed for the differences in cyclization pathways of **1b** that have been observed with potassium hydroxide and potassium methoxide.

The present results are of particular interest when compared with those obtained by Money, *et al.*,[9] and Crombie and James[10] in studies of basic cleavage of pyranopyrones **4**. These compounds undergo conversion to benzenoid derivatives, presumably *via* triketodicarboxylic acids (or esters) **5**. Under most conditions aldol-type cyclizations occur to give β-resorcylic acid derivatives **2** (and **6**). In contrast, with excess magnesium alkoxides Claisen condensations occur to give phloroglucinol derivatives **7**. Money, *et al.*, suggested the latter reaction as a possible model of the biogenetic process for formation of acylphloroglucinols.[9] Crombie and James have presented evidence that the specific catalysis by excess magnesium ion may be caused by chelation of two magnesium ions with each molecule of **5**, with one of these chelation sites involving the 4-carboxyl group.[10]



While in no way trying to detract from the synthetically and theoretically important results obtained by the previous investigators, it should be noted that in natural systems polyketo acids probably do not have appended carboxyl groups, such as in **5**. Moreover, the acylphloroglucinols and related compounds that have been isolated from plants and microorganisms generally do not contain carboxyl groups, such as in **7**.[11] Consequently, the present cyclization of triketo ester **1b**, even though it was conducted under highly basic conditions, is suggested to be a more satisfactory model of acylphloroglucinol biosynthesis than are the reactions of **5**.

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(11) The alternative conversion of pyranopyrones to β-resorcylic acid derivatives is not subject to this criticism, since loss of the 4-carboxyl group of **5** apparently occurs, leading to the natural resorcylic acids. Money, *et al.*, have reported the preparation by this method of the metabolites orsellinic acid, pinosylvin, dihydropinosylvin, and 2-acetonyl-4,6-dihydroxybenzoic acid.[9]

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