tion (4.2 Hz) with the 4α-proton resonating at δ 3.92 (bs, $W_{\frac{1}{2}}^{1}=7$). Oxidation with pyridinium dichromate in CH_2Cl_2 of heptaacetate **3a** gave a monoketone **3b**, whose ^{1}H NMR-spectrum[8] was devoid of the 4α-H signal and showed the 19-H signal at upfield position, δ 1.00, relative to **3a**, δ 1.22, thus giving evidence for the removal of a 1,3-diaxial methyl-hydroxyl interaction in the conversion **3a** → **3b**, consistent with a 4β-OH assignment in **3a** (and **3**).

The D-configuration of the 2-OMe-xylosyl and the L-configuration of the arabinosyl residues in **3** were established by using the same procedure used with nodososide (**1**)[4] and attenuatoside A-I (**2**)[1].

Acid methanolysis of attenuatoside B-I (**3**) followed by benzoylation with p-bromobenzoyl chloride and pyridine of the reaction mixture and TLC-SiO_2 separation in 30% ethyl ether/light petroleum, b.p. 40–70 °C, gave a) methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-arabinopyranoside, characterized by ^{1}H NMR which is described in the preceding paper[1], whose large positively split CD curve, $\Delta\varepsilon_{253}+90$, $\Delta\varepsilon_{236}-29$, A = +119 indicated that arabinose belong to the L series[9] and b) both the anomeric methyl 2-O-methyl-3,4-di-O-(p-bromobenzoyl)-xylopyranosides, characterized by ^{1}H NMR which are described in the preceding paper[1], whose negatively split CD curves (β-anomer; CD: 236/253, $\Delta\varepsilon+14/-36$, A = −50) established that 2-OMe-xylose belong to the D series[9].

Attenuatoside B-II, $[\alpha]_D-9.0°$ (c, 0.5 MeOH), was assigned the related structure **4** by simply comparing the ^{13}C and ^{1}H NMR-spectra with those of the previous glycoside **3** (tables 1 and 2). Assignements of the sugar carbon atoms have been made by comparing its spectrum with that of methyl-α-L-arabinofuranoside (C-1: 109.2, C-2: 81.8, C-3: 77.5, C-4: 84.9, C-5: 62.4 ppm)[10].

Attenuatoside C (**5**), $[\alpha]_D+4.7°$ (c, 0.5 MeOH), is isomeric with the previous monoglycoside **4**. The ^{1}H NMR-spectrum of attenuatoside C (table 2) was very similar to that of attenuatoside B-II (**4**), the major difference being at C-15 [δ 4.24 ddd (J = 11.0, 11.0, 4.5 Hz) vs 4.45 ddd (J = 6.0, 6.0, 1.8 Hz) and C-18 (0.99s vs 1.29s)]. This clearly suggested the 2 compounds be epimeric at C-15. The ^{13}C NMR of **5** differed from that of **4** at C-7 (51.4 vs 50.3), C-8 (75.3 vs 76.5), C-13 (44.6 vs 43.6), C-14 (66.9 vs 61.7), C-15 (68.9 vs 70.0), C-17 (54.8 vs 57.0) and C-18 (15.5 vs 16.4). The differences observed in the 2 spectra are close to those expected for 2 epimeric 15-hydroxysteroids based on the substituent effects recently published for hydroxysteroids[5,6]. In confirmation, attenuatoside C (**5**, 8β-OH, 15α-OH), unlike attenuatoside B-I (**3**) and B-II (**4**), did not react with phenylboronic anhydride. Indeed both the glycosides **3** and **4** formed monophenylboronates[11] in agreement with their 8β-OH 15β-OH stereochemistries.

The arabinose unit in both **4** and **5** belongs to the L series as shown by the large positively split CD curves of the methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-arabinopyranoside (amplitude + 120, in both cases) obtained from the monoglycosides by using the procedure described above.

1 Part 9. L. Minale, C. Pizza, R. Riccio and F. Zollo, Experientia *39* (1983) 567. This contribution is part of the Progetto Finalizzato 'Chimica fine e secondaria' del C.N.R., Roma.

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7 The heptaacetate **3a** was prepared by using an excess of acetic anhydride in pyridine at room temperature, ^{1}H NMR (270 MHz, $CDCl_3$), δ 0.86 (d, J = 6.7 Hz, 26- and 27-H), 0.93 (d, J = 7.0 Hz, 21-H), 1.22 (s, 19-H), 1.29 (s, 18-H), 2.03, 2.09 and 2.10 (singlets, 21H, $CH_3C=O$), 3.31 (m, overlapping with 5″-Hax, 24-H), 3.92 (bs, $W_{\frac{1}{2}}=7$ Hz, 4α-H), 4.66 (dt, J = 11.0 and 4.2 Hz, 3α-H), 5.12 (m, overlapping with 3″-H, 15α-H), 5.40 (ddd, J = 10.5, 10.5 and 3 Hz, 6β-H).

8 **3b**, ^{1}H NMR (270 MHz, $CDCl_3$), δ 0.86 (d, J = 6.7 Hz, 26- and 27-H), 0.93 (d, J = 7.0 Hz, 21-H), 1.00 (s, 19-H), 1.21 (s, 18-H), 2.03, 2.10, 2.11, 2.14 (singlets, 21H, $CH_3C=O$), 2,47 (d, J = 10.5, H-5), 3.31 (m, overlapping with 5″-Hax, 24-H), 5.10–5.20 (m, overlapping with 3″-H and 5′-H, 3α-H and 15α-H), 5.52 (ddd, J = 10.5, 10.5 and 3.0 Hz, 6β-H).

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11 The monophenylboronates of **3** and **4** were characterized by ^{1}H NMR-spectroscopy: **3**, δ 1.11 (3H, s, 18-H), 1.48 (3H, s, 19-H), 4.27 (1H, ddd, J = 10.5, 10.5, 4.0 Hz, 6β-H), 4.68 (1H, brt, J = 5.5 Hz, 15α-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: δ 7.35 (3H, m), 7.75 (2H, d, J = 7.5 Hz); **4**, δ 1.11 (3H, s, 18-H), 1.48 (3H, s, 19-H), 4.27 (1H, ddd, J = 10.5, 10.5, 4.0 Hz, 6β-H), 4.68 (1H, brt, J = 5.5 Hz, 15α-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: δ 7.35 (3H, m), 7.75 (2H, d, J = 7.5 Hz).

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Formation of microspheres from simple molecules under simulated primitive earth conditions; ultrasound and light radiation

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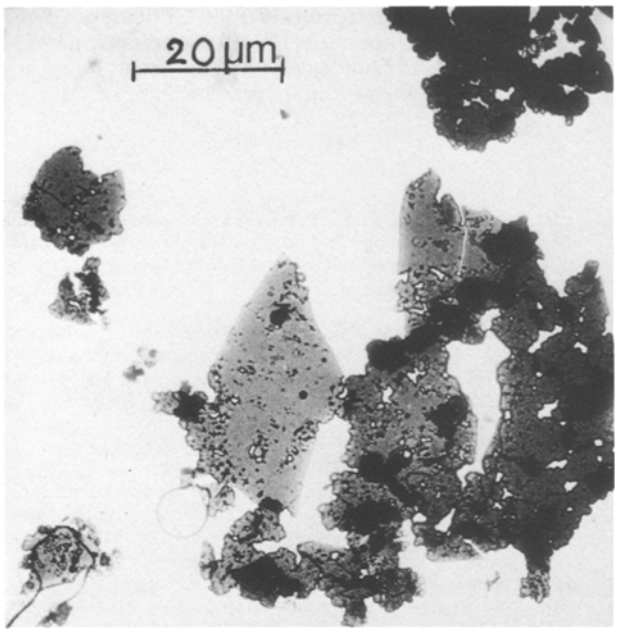
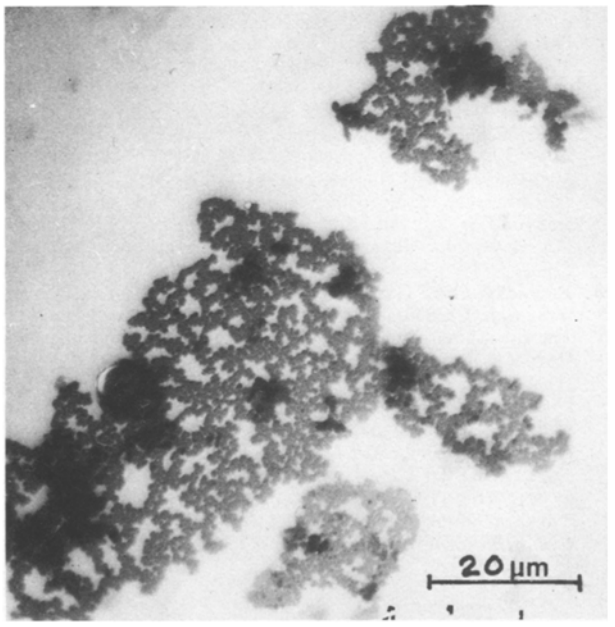
Summary. We have synthesized microspheres from aqueous solutions of simple molecules submitted to sonolysis at 20 kHz and 800 kHz in an argon atmosphere. Photochemical reaction under room light or UV-irradiation yielded the same microstructures, whose formation has been studied with optical and electronic microscopes.

For a number of years, it has been known that microspheres or microspherules are formed when simple molecules are exposed to the action of heat, UV-radiation or spark discharge. Fox and Dose[2], Labadie et al.[3], Yanagawa and Egami[4] synthesized microspheres by means of exposure to heat. Using formaldehyde as the starting material,

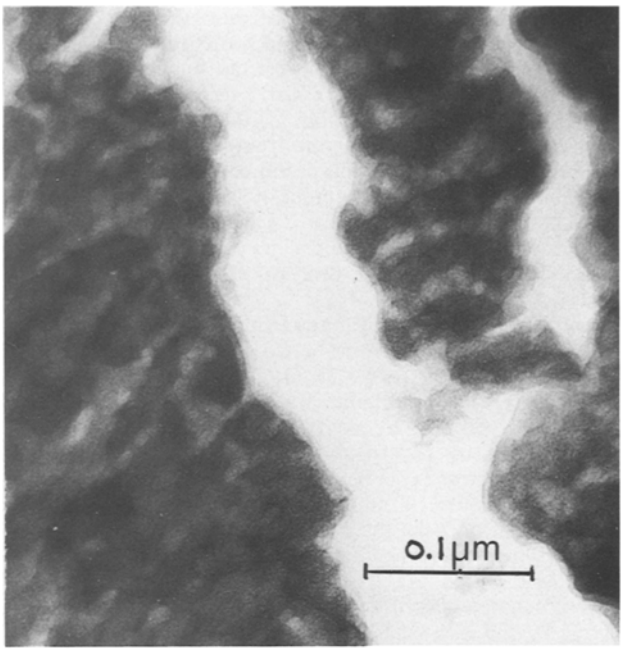


a

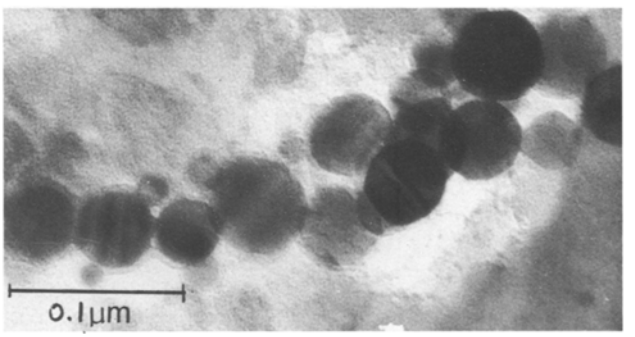


b

Figure 1. Optical micrograph. *a* Transformation of the hydrophobic film; *b* occurrence of microspheres.



a



b

Figure 2. Transmission-electron micrograph. *a* Wrinkled film; *b* occurrence of microspheres.

Bahadur[5] and Smith et al.[6] produced organic microstructures by the action of light. Grossenbacher[7], Folsome[8], Simionescu et al.[9] utilized spark discharge in a gas mixture. Pollock and Heiderer[10] prepared microspheres of aldocyanoin without any external source of energy. The present study deals with the synthesis of microspheres by the use of another source of energy, namely ultrasound (US).

Experimental procedure. We used Bahadur's solution[5] which consists of equal volumes of the following 2 aqueous solutions:

1. 1.26 M diammonium hydrogen phosphate, 0.017 M ammonium molybdate and 1% (by weight) each of: NaCl, K_2SO_4, $Ca(CH_3CO_2)_2$, $MgSO_4$, the whole being adjusted to pH 2 with HCl.
2. Formaldehyde, 36%. This concentration of formaldehyde precludes any microbial contamination in the resulting solution.

Two frequencies of ultrasonic radiation were used, for about 20 h each. The experimental solution was protected from light and saturated with argon.

1. 20 kHz. A Branson generator, coupled to a vibrating transducer, immersed in 300 ml of solution, operated at 20 kHz. Since the cell could not be hermetically sealed, argon was gently bubbled through the solution during the experiment.
2. 800 kHz. A Lutzezia generator, coupled to a barium titanate transducer, operated at 800 kHz. A 150 ml glass-stoppered cell, whose bottom consisted of a 0.03 mm nitrocellulose film, transparent to US[11], was filled with 90 ml of solution. We had to operate at low power to avoid the physically destructive effects of US during the formation of particles. During sonolysis, the gas had to be evacuated to prevent a pressure build-up in the cell, as a result of the evolution of formaldehyde.

With both ultrasonic frequencies, we invariably observed the formation of colored microstructures in the solution, which were separated by filtration, promptly washed with water and dried in a dessicator. After being dried, they are insoluble in most solvents. The sizes of these blue-green spherical particles varied from 1 to 10 µm in diameter.

Analysis results and discussion. Elemental analysis of the microspheres yielded the following results:

	C%	N%	H%	atomic ratio C/N
20 kHz irradiation	12.22	7.95	2.49	1.80
800 kHz irradiation	9.75	7.23	3.65	1.57

ash about 70%

After prolonged hydrolysis in 6 N HCl and evaporation of excess acid, the microspheres were subsequently subjected to TLC. The hydrolysates were first tested for the presence of amino acids, using cellulose plates, and n-butanol/acetone/diethylamine/water (10:10:2:5) as solvent[12]. The chromatograms were sprayed with ninhydrin, 2-4-6 collidine, acetic acid mixture[13] and the spots of glycine, α and β alanine, glutamic acid and a prominent unidentified violet spot were observed, but the amount of amino acids was moderate.

We searched unsuccessfully for nucleic acids, sugars and lipids. In aqueous suspension, the microspheres turned the water blue, but their shapes were unchanged, except that some particles were partially discolored. The resulting solution contained soluble compounds which yielded chromatograms similar to those of the microspheres.

An IR-spectrum showed a strong absorption at 2850-2920 cm^{-1} which indicated the presence of compounds rich in $-CH_2-$and$-CH_3$, but the characteristic bands of the peptide bond were not found. Mass spectrometry, by the desorption, chemical ionization technique, with the NH_4^+ion, gave the following results:

m/e 44, 46, 59, 60, 73, 74, 77, 83, 87, 91, 98, 100, 103, 108, 114, 120, 128. Some of these values can be attributed to the protonated ions of glycine, alanine and methyl alanine or their fragmentation products, but heavier ions were not observed.

The analytical results suggest that the microspheres consist of a soluble derivative of amino acids mixed with an insoluble polymer, both containing molybdenum and other mineral elements.

To compare the synthesis described above with Bahadur's photochemical method, we exposed similar solutions to room light and UV-radiation, respectively. With room light, the UV is absorbed by the glass walls of the sealed flask containing the solution, which becomes slowly greenish. Microspheres appear after several weeks. When a solution, protected from evaporation by a quartz cover, is irradiated by a mercury lamp ($\lambda = 253{,}7$ nm), it turns blue and a rapid growth of microspheres is observed.

Analysis of these colored particles gives the same results as those previously obtained with US, whereas Bahadur[5] found more amino acids than we do, as well as a large number of other organic compounds. In these syntheses, we tried unsuccessfully to modify the composition of the solution; only the mixture noted above yields stable microstructures.

The formation of particles, under UV-radiation, has been observed with optical and electronic microscopes (figs 1 and 2). First the surface of the solution becomes coated with a hydrophobic film, made up of blue patches; subsequently the film wrinkles, breaks up and is finally transformed into microspheres.

In this paper, we have shown that sonolysis, like other sources of energy, can produce microparticles. Such structures could have been formed during the chemical evolution of early prebiotic times.

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The effect of a gastric mucus barrier on the dynamic response of a pH electrode

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Summary. Hydrogen ion mobility in gastric mucus has been found to be reduced to a greater extent than that hitherto suspected, though at low pH (< 4) and in buffer this mobility increases. Mucus, at an optimized pH, may therefore protect the gastric mucosa from acid digestion by providing a diffusion barrier.

The thin layer of mucus which lines the gastric mucosa has long been thought to be a defence against the damaging effects of acid gastric juice. The rate of H^+ diffusion, previously observed to be as great as that in water[2], has recently been shown to be reduced in mucus[3,4]. The diffusional resistance has been attributed in turn to the gel-like nature of mucus, with its ability to immobilize water molecules, and to a possible steric hinderance from the mucus glycoprotein itself. Undoubtedly gastric mucus can maintain steep pH gradients in acid if left in contact with the gastric epithelium[5-7] but the capability has been considered mainly to be due to the neutralizing action of bicarbonate secreted into mucus by the surface epithelial cells[8] rather than to the effect on diffusion. We have examined H^+, Na^+ and K^+ mobility in isolated pig gastric mucus using glass ion-selective electrodes. Our study shows that