Confirmation of these results was provided by experiments conducted in Helsinki with Jensen's culture K of *A. vinelandii*, the same strain of bacteria employed in the original study by Virtanen and Lundbom [4]. Cultures were supplied nitrogen either as 0.2 atm. normal N_2, 50 ppm ammonium nitrogen (7 atom per cent ^{15}N excess), or 500 ppm nitrate nitrogen (7 atom per cent ^{15}N excess). The test flasks were incubated in desiccators at 30° under 0.2 atm. N_2, 0.2 atm. O_2, and either 0.6 atm. N_2O or 0.6 atm. vacuum. The cells were harvested at 3, 8, 19, and 24 hours after inoculation. Total cellular nitrogen was determined, and the cells exposed to $^{15}NH_4^+$ or $^{15}NO_3^-$ also were analyzed for ^{15}N. The results of these experiments are summarized in Table 2; the results for total nitrogen on cultures furnished $^{15}NH_4^+$ and $^{15}NO_3$ are not recorded in the table, but they gave a picture entirely comparable to the ^{15}N analyses listed. These data further substantiate the specificity of N_2O inhibition for nitrogen fixation. It is clear that *A. vinelandii* using combined nitrogen either as ammonia or nitrate, is not inhibited by nitrous oxide.

1. Molnar, D. M., Burris, R. H. and Wilson, P. W. *J. Am. Chem. Soc.* **70** (1948) 1713.
2. Repaske, R. and Wilson, P. W. *J. Am. Chem. Soc.* **74** (1952) 3101.
3. Wilson, T. G. G. and Roberts, E. R. *Biochim. et Biophys. Acta* **15** (1954) 568.
4. Virtanen, A. I. and Lundbom, S. *Acta Chem. Scand.* **7** (1953) 1223.

Received July 14, 1955.

Studies on the Chemistry of Lichens. I. D-Arabitol from *Alectoria jubata* Ach., var. *chalybeiformis* Th.Fr.

YNGVE JOHANNES SOLBERG

Universitetets Kjemiske Institutt, Blindern-Oslo, Norway

In the course of a series of investigations on lichen substances in the genus *Alectoria*, the *Alectoria jubata* var. *chalybeiformis* has been studied.

Only one lichen substance, D-arabitol, was obtained by extraction of the lichen with acetone. It crystallized very slowly from saturated solutions. Occasionally the substance precipitated as an oily product from acetone or alcohol and solidified to a crystalline mass on standing or by scratching.

An authentic sample of D-arabitol was not available. The specific rotation from saturated, aqueous boric acid solution was +7.66°, in fair agreement with the value of +7.82° reported by Asahina [1].

X-ray diffraction patterns of the substance and of L-arabitol were identical.

Experimental. Air-dried, ground *Alectoria jubata* var. *chalybeiformis* (208 g) (collected by cand.real. Eilif Dahl, on birch in Rondane, Norway) was continuously extracted with acetone for twenty hours.

The greater part of the solvent was removed by distillation, and on standing in a refrigerator the solution deposited colourless crystals (A). These were separated, washed with ether and dried. The dark green filtrate was concentrated to a small volume and the viscous residue repeatedly treated with cold alcohol. Undissolved material was removed by filtration (B). A and B both appeared to be the same substance and were purified together by recrystallization from acetone. After having been kept at —22° for some ten days 0.32 g of

Table 1. Spacing values in Å. (w = weak, m = medium, s = strong, vs = very strong.)

Substance from *Alectoria jubata* var. *chalybeiformis*	L-arabitol
7.37 s	7.37 s
5.48 »	5.48 »
4.83 vs	4.83 vs
4.63 »	4.60 »
4.33 »	4.33 »
3.96 w	3.95 w
3.60 vs	3.61 vs
3.42 s	3.42 s
3.23 »	3.23 »
3.12 »	3.11 »
2.83 m	2.83 m
2.52 s	2.52 s
2.41 w	2.42 w
2.37 »	2.38 »
2.15 s	2.16 s
2.08 »	2.09 »
2.03 w	2.03 w

the substance was obtained. After washing with ether and recrystallization from ethanol-petroleum ether in 1:1 ratio 0.16 g of pure D-arabitol was obtained; m. p. 100° (uncorr.). (Found: C 39.54; H 8.06. Calc. for $C_5H_{12}O_5$: C 39.47; H 7.95.) $[\alpha]_D^{21}$ + 7.66° (from saturated, aqueous boric acid solution, c = 2.2).

The comparison of X-ray diffraction patterns of L-arabitol and the substance from *Alectoria jubata* var. *chalybeiformis* crystallized from acetone gave the spacing values in Å, recorded in Table 1. The instrument used was one Philips X-ray Geiger Counter Spectrometer with Cu-$K\alpha_1$ radiation.

L-Arabitol was kindly provided by Farmasöytisk Institutt, Blindern-Oslo, Norway.

1. Asahina, Y. and Shibata, S. *Chemistry of Lichen Substances*, Japan Soc. for the Promotion of Science, Tokyo 1954.

Received August 22, 1955.

Sialic Acid in Human Cervical Mucus, in Hog Seminal Gel, and in Ovomucin

LARS ODIN

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

The presence of sialic acid as a characteristic constituent in epithelial mucus has been indicated by colour reactions [1]. This and/or closely related substances have also been isolated in crystalline form from certain materials of epithelial origin [2-8]. The isolation of a sialic acid from three additional similar sources, *viz.* human cervical mucus, the gelatinous lumps of hog semen, and ovomucin, will be reported here.

Using quantitative colorimetric methods Werner [1] has found, that the mucous plugs of the pregnant human uterine cervix contain considerable amounts of sialic acid in addition to glycoprotein material of the same general composition as the blood-group substances.

The carbohydrate components of ovomucin, the probably non-homogeneous, water-insoluble glycoprotein of hen's eggs, have been reported to be galactose and mannose in approximately equimolecular amounts [9], together with glucosamine and galactosamine [10]. By means of colour reactions the presence in ovomucin of fairly large amounts of substance of the sialic acid type has been indicated [10,11].

In hog semen a voluminous gel is formed shortly after ejaculation. No recent investigations on the composition of this material seem to have been published. From human seminal plasma Freudenberg *et al.*[12] have prepared a product showing the properties of the blood group substances. The preparation from the same material of an electrophoretically homogeneous glycoprotein, containing hexosamine but not uronic acid, has also been reported [13].

Experimental. Analytical methods. The same methods as those recently reported [8] were used. Separation of the aminosugars was performed by Gardell's method [14]. The single monosaccharide components in the glycoprotein hydrolysates were determined after paper-chromatographic separation. The X-ray powder diagrams were taken with a Guinier camera using copper $K\alpha$-radiation.

Materials. The cervical mucous plugs were collected immediately before delivery, and stored in 60 % aqueous ethanol. When enough material was obtained the plugs were cut up with scissors and kept in about 0.005 N hydrochloric acid for 24 hours at 4° C. After washing with aqueous ethanol the material was dried with ethanol and ether. The dry powder contained 9.7 % nitrogen, 6.6 % glucosamine, 3.9 % galactosamine, 7.5 % galactose, 5.0 % fucose, and 7.4 % sialic acid.

The colourless gelatinous mass from two ejaculates from a boar was thoroughly washed with saline and distilled water after decanting off the fluid seminal plasma. It was then cut in small pieces, treated with hydrochloric acid, and dried exactly as the cervical mucus. The analysis gave the following composition: 10.7% nitrogen, 2.0 % glucosamine, 8.5 % galactosamine, 2.7 % galactose, 1.3 % mannose, 0.7 % fucose, and 14.7 % sialic acid.

The ovomucin was prepared mainly according to the method of Gottschalk and Lind [15]. To cold homogenized egg-white, freed from chalazae by passing through a filtering cloth, cold distilled water was added. The mixture was centrifuged, and the gelatinous precipitate obtained dispersed in 10 % sodium chloride and reprecipitated by addition of distilled water. The precipitate was washed with saline and distilled water until the washings were free from protein. The material was then treated with hydrochloric acid, and dried in the