Comments and Communications

Reaction of Bromine Water with Alfalfa Saponins

The recent report that alfalfa saponins are involved in bloat of ruminants has created interest in this group of compounds (*1*). Investigations in our laboratory have been centered on a mixture of saponins isolated from alfalfa by Walter *et al.* (*2*). No pure glycosidic compound has been isolated from this mixture, and paper chromatographic techniques have failed to resolve it into its component parts.

During a cursory examination of the effect of various oxidizing agents on alfalfa saponins we observed that saturated bromine water reacts rapidly with this mixture at room temperature, as evidenced by the disappearance of the bromine color, by the formation of a white precipitate, and by paper chromatographic studies.

Examination of the filtrate from the bromine water treatment by paper chromatography (*3*) revealed three spots on the chromatogram, all of which moved more slowly than the control sugars glucose, arabinose, xylose, and rhamnose. The rate of movement of the three spots suggested an oligosaccharide type of material and the spots gave the characteristic pentose-color reaction with the aniline spray reagent.

When untreated alfalfa saponins are hydrolyzed for two hours with 1*N* hydrochloric acid in a sealed tube in a boiling water bath, paper chromatography always reveals glucose, arabinose, xylose, and rhamnose on the chromatogram. When each of the three carbohydrate fractions obtained from the bromine-treated saponin was hydrolyzed similarly with 1*N* hydrochloric acid, the two slowest fractions were each composed of arabinose, xylose, and rhamnose. These two fractions contain the same sugars, but preliminary studies have indicated that the molecular ratios of the component sugars may be different. The third and fastest fraction consisted of glucose, arabinose, xylose, and rhamnose. The experimental data thus indicate that the bromine water cleaves sugars from the alfalfa saponins in the form of three sugar polymers. It seems appropriate to report these findings at this time because of Potter and Kummerow's (*4*) recent report on the isolation of three triterpene genins from dehydrated alfalfa leaf meal.

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Free Amino Groups of Human Serum Albumin

It has been reported by Brand and Van Vunakis (*1*), using Sanger's DNP technique (*2*), and it is confirmed in this paper that there is one free alpha amino group, belonging to aspartic acid, in human serum albumin. The same free amino group was also found in bovine serum albumin by Brand and Van Vunakis.

McClure, Schieler, and Dunn (*3*), using Edman's phenyl isothiocyanate technique with bovine albumin, however, found a number of terminal alpha amino groups, of which two or three were contributed by aspartic acid, one by methionine, one by histidine, and an undetermined number by alanine.

Weber has observed an increased availability of the terminal groups of bovine serum albumin in methyl ethyl ketone. This is demonstrated by an increased rotational freedom of groups in the molecule, presumably about many bonds (*4*). It was postulated that the increased rotational freedom may make accessible to Sanger's fluorodinitrobenzene reagent groups that would otherwise be masked, and that these additional groups might account for the other free alpha amino groups found by McClure *et al.*, but not found by Brand and Van Vunakis.

It was of interest to know whether this might be true for human serum albumin. The DNP reaction was carried out first in the usual way as described by Sanger (*2*) by adding 1 g of 1:2:4 fluorodinitrobenzene (FDNB) in 10 ml of 95-percent ethyl alcohol to 0.5 g of human serum albumin dissolved in 5 ml of 10-percent sodium bicarbonate, *p*H 8.5. The mixture was shaken for 2 hr. The insoluble DPN albumin was washed three times each with water, alcohol, and ether, and then it was air-dried. A second sample was prepared in the same manner except for the fact that the FDNB was dissolved in 10 ml of methyl ethyl ketone, and the albumin was dissolved in 5 ml of a 10-percent solution of sodium carbonate, sodium bicarbonate, *p*H 9, the *p*H at which McClure's group worked. One hundred milligrams of air-dried DNP albumin were found by Thompson (*5*) to contain 79 mg of moisture-free albumin by amide determinations, and these figures were used here. Fifty-milligram samples of each preparation were hydrolyzed in 5.7*N* HCl by boiling under reflux for 4 hr and for 24 hr. DNP amino acids were identified by silica gel and filter paper chromatography (*2, 6*), the silica gel chromatography being quantitative. Control experiments showed that there was a 65-percent breakdown of DNP aspartic acid during 24 hr of hydrolysis. The 15-percent breakdown in 24 hr for the epsilon DNP lysine used was that reported by Sanger (*2*).

The results were the same in aqueous methyl ethyl ketone as in aqueous ethanol: one alpha amino group of aspartic acid (0.365 and 0.345 after 24-hr hydrolysis, corrected to 1.04 and 0.98, respectively) and