traces of natural photosensitizers in the phage preparation[10].

Under the same experimental conditions, however, SP 8 phages remain unchanged as well as the dark controls of both SP 8 and SP 3 phages in PBS supplemented with the dyes. Neither SP 8 nor SP 3 phage were inactivated after 2 h of dark treatment with 1/100,000 trypaflavine in PBS at neutral pH.

Photodynamic action of riboflavine (see Table) which is only rarely observed in the phage inactivation recalls the similar findings of GALSTON and BAKER[11] and GALSTON[12] on T_{2r} and T_{6r} phages of *Escherichia coli*.

The present results of our experiments on the photodynamic inactivation of serologically unrelated SP 8 and SP 3 phages of *B. subtilis* can be summarized as follows: (a) SP 3 phage is much more sensitive to the photodynamic action than is SP 8 phage; (b) the rate of the photodynamic inactivation of both phages mentioned above depends strongly on the chemical structure of the photosensitizing dye used in the experiment.

Hence the above results are seen to be generally in accord with the conclusions of BURNET[3] and WELSH and ADAMS[4] as regards a known correlation between the serological grouping of some coli-bacteriophages, which is of taxonomic significance, as well as between the chemical structure and photodynamic activity of various photosensitizing organic dyes on the T-group of coli-phages described by YAMAMOTO[5] and KAUFMAN and HIATT[6].

Zusammenfassung. Es wurde die photodynamische Aktivität von 8 verschiedenen organischen Farben auf zwei Bakteriophagen SP 3 und SP 8, virulent für *Bacillus subtilis*, mittels Inaktivationskonstanten der ersten Ordnung verglichen. Die grösste photodynamische Wirkung zeigt Methylenblau; Neutralrot und Eosin sind praktisch inaktiv.

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Chemical Investigation of the Seeds of *Albizzia procera* Benth[1]

Albizzia procera Benth, commonly known as 'safed siris' in Hindi, is widely distributed in India. VARSHNEY et al.[2,3] reported the isolation of machaerinic acid[4] and proceric acid[5,6] from the seeds of *A. procera* Benth. From the seeds of *A. procera* collected in the Jalpaiguri District, West Bengal, we have obtained an amount of saponin which on hydrolysis gave a mixture of acid and neutral sapogenins. The crude acid sapogenin, on treatment with diazomethane and subsequent column chromatography on alumina, gave 3 colourless crystalline fractions A, B and C.

Fraction A was found to be identical with methyl machaerinate[4].

Fraction B, $C_{30}H_{46}O_4$, m.p. 294–296°, $[\alpha]_D^{26}$ −13° ($CHCl_3$), gave a purple-violet coloration in the Liebermann-Burchard test and a pale yellow colour with tetranitromethane. The IR-spectrum in KBr pellet showed characteristic bands at 3550 cm^{-1} (hydroxyl group), 1765 cm^{-1} (a 5-membered lactone) and at 1360 and 1380 cm^{-1} (*gem*-dimethyl groups). Fraction B afforded a crystalline monoacetate, $C_{32}H_{48}O_5$, m.p. 301–304°, on treatment with pyridine and acetic anhydride at 0°. The IR-spectrum of the monoacetate in KBr pellet showed characteristic bands at 3650 cm^{-1} (hydroxyl group), at 1720 cm^{-1} and 1240 cm^{-1} (acetoxyl carbonyl), and at 1370 and 1385 cm^{-1} (*gem*-dimethyl groups). Fraction B, and also the above monoacetate on heating with pyridine and acetic anhydride on a steam-bath, gave a diacetate, $C_{34}H_{50}O_6$, m.p. 246–248°, $[\alpha]_D^{26}$ −38° ($CHCl_3$). It appeared to be a new compound and was named proceragenin A.

Fraction C, $C_{30}H_{46}O_4$, m.p. 255–258°, $[\alpha]_D^{31}$ +6.53° ($CHCl_3$), also responded to the Liebermann-Burchard test (purple-violet) and gave a pale yellow colour with tetranitromethane. The IR-spectrum showed bands at 3500 cm^{-1} (hydroxyl), 1765 cm^{-1} (a 5-membered lactone), and at 1360 and 1380 cm^{-1} (*gem*-dimethyl groups). It formed a diacetate, $C_{34}H_{50}O_6$, m.p. 238–240°. The physical and chemical properties of this fraction did not agree with any known compound and was named proceragenin B.

The crude neutral sapogenin on chromatographic resolution over alumina gave 5 crystalline products D, E, F, G and H.

Fraction D, m.p. 130–140°, a minor constituent, was found to be a mixture of sterols.

Fraction E, $C_{30}H_{46}O_3$, m.p. 265–268°, $[\alpha]_D^{32}$ −51.2° ($CHCl_3$), gave a purple-violet coloration with acetic anhydride and concentrated sulphuric acid and a pale yellow colour with tetranitromethane. The IR-spectrum showed bands at 3450 cm^{-1} (hydroxyl group), 1775 cm^{-1} (a 5-membered lactone), and at 1367 and 1380 cm^{-1} (*gem*-

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dimethyl groups). It furnished a monoacetate, $C_{32}H_{48}O_4$, m.p. 300–305°, $[\alpha]_D^{32}$ — 26.4° ($CHCl_3$). Fraction E on hydrolysis with alcoholic caustic potash furnished machaerinic acid[4]. Formation of machaerinic acid suggests fraction E to be 28 → 21 lactone of 3β:21β-dihydroxy-olean-12-ene-28-oic acid with conformational alternation[7,8] in ring D or E, since the steric requirement for 28 → 21 lactonization is not fulfilled in *cis*-locked D/E rings with all chair conformation.

Fraction F, $C_{32}H_{52}O_4$, m.p. 198–199°, $[\alpha]_D^{31}$ + 81° ($CHCl_3$), gave a diacetate, $C_{36}H_{56}O_6$, m.p. 223–224°, $[\alpha]_D^{32}$ + 85.45° ($CHCl_3$). The IR-spectrum showed bands at 3400 cm^{-1} (hydroxyl), 1710 cm^{-1} (ester carbonyl), and at 1365 and 1370 cm^{-1} (*gem*-dimethyl). Fraction F responded to the characteristic test of a triterpene in the Liebermann-Burchard reaction and in tetranitromethane, and was identified as ethyl ester of machaerinic acid. This acid, on treatment with diazoethane, gave Fraction F. The latter was proved to be formed during isolation.

Fraction G, $C_{30}H_{46}O_4$, and Fraction H, $C_{30}H_{48}O_4$, were found to be identical with proceragenin A and proceragenin B respectively obtained from the acid sapogenin fraction. Proceragenin A and proceragenin B on hydrolysis with alcoholic caustic potash yielded acids, which on treatment with diazomethane or on standing with alcoholic hydrochloric acid gave back proceragenin A and proceragenin B respectively. This is perhaps the reason why these compounds are encountered from both the acid and neutral sapogenin fractions during isolation.

Further work on the constitution of proceragenin A and proceragenin B is under progress.

Zusammenfassung. Aus dem Samen von *Albizzia procera* Benth wurden ausser Machaerinsäure 4 triterpenoide Sapogenine isoliert (Verbindung A und B, Proceragenin A und B). Es wurde bewiesen, dass die Verbindung A das 28 → 21-Lacton der Machaerinsäure mit einer Konformationsänderung am Ring D oder E ist, während die Verbindung B als Äthylmachaerinat identifiziert wurde. Bei Proceragenin A und B handelt es sich um Dihydroylactone.

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The Indigogenic Reaction for Histochemical Demonstration of Alkaline and Acid Phosphatase

Previous work in this laboratory described the application of the indigogenic principle to the histochemical demonstration of leucine aminopeptidase[1], β-glucosidase[2], β-galactosidase[3] and N-acetyl-β-glucosaminidase[4]. In the present study we have extended this principle to specific localization of alkaline and acid phosphatase, utilizing 5-bromo-4-chloro-3-indolyl phosphate (I) and 5-bromo-6-chloro-3-indolyl phosphate (II) (as the para-toluidine salts) as substrates. Both products were synthesized according to methods described recently by HORWITZ and co-workers[5,6]. The substrates offer the advantage of a precise enzyme localization with no or very slight diffusion. Moreover, the substrates afford a simple and direct method for demonstration of hydrolytic enzymes without the need for a coupling reaction. Compound I yields a blue-green deposition of indigo whereas compound II gives a magenta colored precipitate.

Methods. Tissues from mouse, rat, guinea-pig and man were used for this study. Representative pieces of tissue from each organ were removed and cut into blocks 2–4 mm in thickness, and quick-frozen by placing the tissue in a glass tube and immersing it in a Dewar flask containing acetone and dry ice at — 70 °C. The tissues were embedded in O.C.T. (optimal cutting temperature) compound, purchased from Lab-Tek, composed of water-soluble glycols and resins matched to a specific cutting zone temperature of — 20 °C to — 35 °C. The embedded tissue was then placed on the quick-freeze bar of a Lab-Tek cryostat for 1 min until the embedding medium was frozen and became the proper consistency for cutting 6 μ sections at — 20 °C. After cutting, the sections were attached to warm slides and fixed for 5 min in cold acetone, passed through 95% and 85% acetone and then rinsed in distilled water. All solutions were maintained at 4 °C for preservation of enzymatic activity. The slides were then air dried to prevent the formation of ice crystals and stored at — 25 °C until incubated in the specific substrate solution. Fresh frozen and cold acetone-fixed sections were incubated for 2 h in solutions containing para-toluidinium 5-bromo-4-chloro-3-indolyl phosphate or para-toluidinium 5-bromo-6-chloro-3-indolyl phosphate, depending on the desired color of the end-product. The solutions were made specific for acid and alkaline phosphatase by varying the pH of the buffer used for incubation. The incubating solution employed for alkaline phosphatase is as follows: 14.0 ml *Tris* buffer pH 8.4–9.3, 0.05 *M*; 1.0 ml para-toluidinium 5-bromo-4-chloro-3-indolyl phosphate or para-toluidinium 5-bromo-6-chloro-3-indolyl-phosphate (2.1 mg/ml of dimethylformamide), 0.00031 *M* (final concentration); 1.0 ml $MgCl_2$ 0.005 *M*

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