

Action du DCP seul. En dehors de son intervention dans le mécanisme de la réaction AIA-oxydasique, tel qu'il est proposé par YAMASAKI et PIETTE² par exemple, il est clair que le DCP lève l'inhibition due à des substances naturelles, dont la synthèse est favorisée par la lumière^{5,8}. L'effet inhibiteur du DCP en grande concentration peut être attribué à son hydroxylation, par les peroxydases, en forme polyphénolée inhibitrice de la destruction auxinique¹².

Action combinée de H₂O₂ et du DCP. Les interactions entre DCP et H₂O₂ décrits plus haut rappellent celles qui sont dues à l'ion Mn⁺⁺ en présence de DCP^{13,14}: à faibles concentrations de phénol (1 · 10⁻⁷ M) l'ion manganèse inhibe le catabolisme auxinique; lorsque le phénol est en concentration plus grande (1 · 10⁻⁴–1 · 10⁻³ M), le manganèse accélère la dégradation de l'AIA. Sans doute existe-t-il des lieux communs entre ces deux types d'interaction et plusieurs causes doivent être recherchées au niveau des mécanismes de la réaction, influencés par ces substances, et qui mettent en jeu la production de radicaux libres, d'eau oxygénée¹⁵, de formes plus ou moins oxydées ou réduites de la peroxydase.

Summary. DCP increases IAA destruction by both *Lens* and *Phaseolus* root breis. H₂O₂ inhibits catabolism by *Lens* extracts but activates it when *Phaseolus* is used, mainly when roots are cultivated in the dark and contain inhibitors of IAA destruction. DCP 1 · 10⁻³ M and H₂O₂ 1 · 10⁻⁴ or 1 · 10⁻³ volume for *Lens* and DCP 1 · 10⁻⁴ M and H₂O₂ 1 · 10⁻³ volume for *Phaseolus* nullify auxin catabolism.

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¹³ W. S. HILLMAN et A. W. GALSTON, *Physiologia Pl.* 9, 230 (1956).

¹⁴ P. E. PILET, *Experientia* 13, 35 (1957).

¹⁵ M. BASTIN, TH. GASPARD et C. LEYH, *C. r. Acad. Sci. Paris* 260, 4050 (1965).

Photodynamic Inactivation of Some *Bacillus subtilis* Bacteriophages

Bacteriophages active against *B. subtilis* isolated by ROMIG and BRODETSKY¹ were recently characterized² on the basis of the following properties: plaque morphology, stability, host range, adsorption kinetics, one-step growth characteristics, calcium requirements, serum neutralization, thermal inactivation and inactivation by UV-light. It was BURNET³ who found some years ago that the relative sensitivity of some coli-phage strains to photodynamic inactivation was correlated with serological grouping and hence was of taxonomic significance. On the basis of the above finding, as well as other related studies⁴⁻⁶, I have recently described the photodynamic inactivation of serologically unrelated SP 8 and SP 3 phages of *B. subtilis*^{1,2} caused by methylene blue and visible light under aerobic conditions⁷. The inactivation kinetics of both phages were found to be first order after a short initial lag. As such studies on *B. subtilis* bacteriophages have not been carried out until now, experiments analogous to that done by me with methylene blue were intended to extend these observations also with some other photosensitizing organic dyes, using essentially the experimental procedure described by WELSH and ADAMS⁴. Westinghouse 200 W tungsten lamps at a distance of 50 cm have been used as a polychromatic light source⁷. *B. subtilis* host strains employed in these experiments were Marburg for SP 8 phage and 168 for SP 3 phage. The methods of assay and handling of host bacteria and bacteriophages described in closely pertinent papers^{1,2,8,9} were applied. The photodynamic inactivation curves of both SP 8 and SP 3 phages showed an initial shoulder followed by a linear portion. The relative sensitivities to the photodynamic inactivation were compared by the slopes of the inactivation curves, using their exponential part only. First-order rate constants for photodynamic inactivation in the presence of various photosensitizing organic dyes were calculated for both phages tested.

The results of experiments carried out are shown in the Table. Illumination of phage SP 3 in phosphate buffered saline (PBS) at pH 7.0 without a dye showed also a small inactivation (about 3% of the value calculated for methylene blue) which may be due to the presence of

Photodynamic activity of various organic dyes at pH 7.0 against SP 8 and SP 3 bacteriophages of *Bacillus subtilis*; dye concentration 1/100,000; phage titres 10⁶/ml

Dye	First order inactivation rate constants, k (min ⁻¹)	
	Bacteriophage	
	SP 8	SP 3
Methylene blue	0.57	1.15
Thionin	0.04	0.13
Thioflavine	0.04	0.04
Neutral red	0.00	0.00
Coriophosphine	0.00	0.04
Eosin	0.00	0.00
Trypaflavine	0.04	0.09
Riboflavin	0.00	0.04

¹ W. R. ROMIG and A. M. BRODETSKY, *J. Bact.* 82, 135 (1961).

² A. M. BRODETSKY and W. R. ROMIG, *J. Bact.* 90, 1655 (1965).

³ F. M. BURNET, *J. Path. Bact.* 37, 179 (1933).

⁴ J. N. WELSH and M. H. ADAMS, *J. Bact.* 68, 122 (1954).

⁵ N. YAMAMOTO, *J. Bact.* 75, 443 (1958).

⁶ E. KAUFMAN and C. W. HIATT, *Virology* 9, 478 (1959).

⁷ D. KALÁB, *Acta virol. Prague* 10, 279 (1966).

⁸ J. MARMUR, C. M. GREENSPAN, E. PALEČEK, F. M. KAHAN, J. LEVINE, and M. MANDEL, *Cold Spring Harb. Symp. quant. Biol.* 28, 191 (1963).

⁹ E. PALEČEK, *Folia biol., Praha* 11, 89 (1965).

traces of natural photosensitizers in the phage preparation¹⁰.

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 trypanflavine 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 BAKER¹¹ and GALSTON¹² 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 BURNET³ and WELSH and ADAMS⁴ 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 YAMAMOTO⁵ and KAUFMAN and HIATT⁶.

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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¹⁰ R. WAHL and R. LATARJET, *Annls Inst. Pasteur*, Paris 73, 957 (1948).

¹¹ A. W. GALSTON and R. S. BAKER, *Science* 109, 485 (1949).

¹² A. W. GALSTON, *Science* 111, 619 (1950).

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Chemical Investigation of the Seeds of *Albizia procera* Benth¹

Albizia procera Benth, commonly known as 'safed siris' in Hindi, is widely distributed in India. VARSHNEY et al.^{2,3} reported the isolation of machaerinic acid⁴ 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 machaerate⁴.

Fraction B, C₃₀H₄₈O₄, m.p. 294–296°, $[\alpha]_D^{26} - 13^\circ$ (CHCl₃), 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, C₃₂H₄₈O₅, 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, C₃₄H₅₀O₆, m.p. 246–248°, $[\alpha]_D^{26} - 38^\circ$ (CHCl₃). It appeared to be a new compound and was named proceragenin A.

Fraction C, C₃₀H₄₈O₄, m.p. 255–258°, $[\alpha]_D^{31} + 6.53^\circ$ (CHCl₃), 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, C₃₄H₅₀O₆, 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, C₃₀H₄₈O₃, m.p. 265–268°, $[\alpha]_D^{32} - 51.2^\circ$ (CHCl₃), 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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