

PECTIN TRANS-ELIMINASE

A. D. AGATE, N. P. JAYASANKAR AND J. V. BHAT

Fermentation Technology Laboratory, Indian Institute of Science, Bangalore-12

EVER SINCE the discovery of pectin by Vanquelin in 1790,¹ pectic substances have interested both the 'pure' scientist as well as the technologist. The pectic substances are carbohydrate derivatives of plant origin. They are complex and colloidal in nature and mostly comprise of anhydrogalacturonic acid units linked together in $\alpha(1-4)$ glycosidic linkage to form a chain-like structure of polygalacturonic acid units. While it is true that D-galacturonic acid is the main constituent of pectin, most natural pectins contain about 12% ester methoxyl. A hypothetical pectin with 75% of its carboxyl groups esterified and polygalacturonic acid are depicted in Fig. 1.

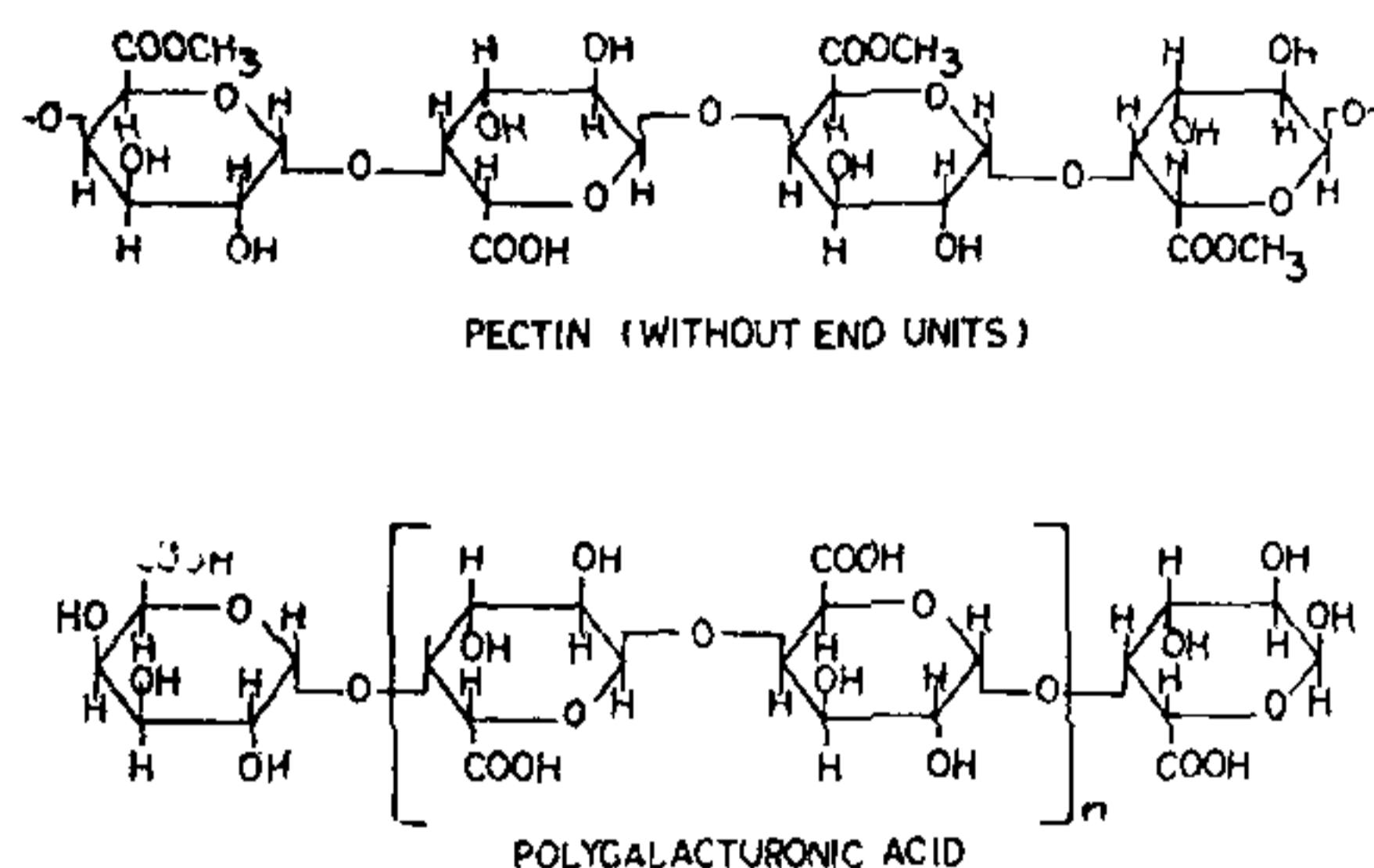


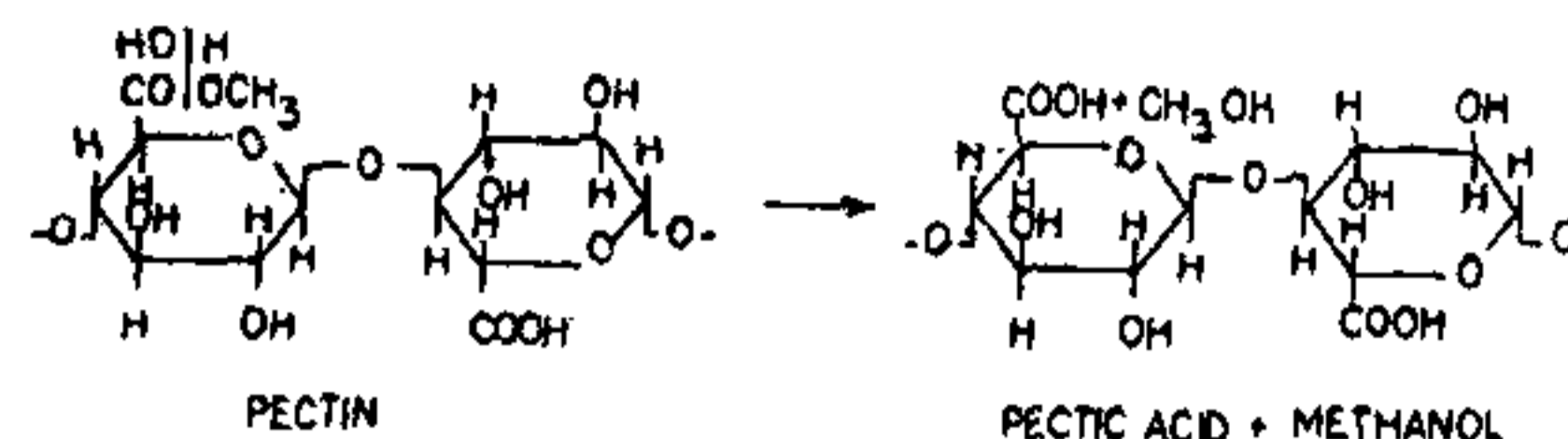
FIG. 1. The Pectic Substances

The complex nature of molecules comprising these substances has not only caused the confusion in their chemistry but has been mainly responsible for the delay in the accumulation of knowledge pertaining to those enzymes which have the power to break these molecules down. However, the immense importance of pectic substances and their enzymes has helped to maintain the tempo of work thereon and it is the purpose of this review to briefly trace the literature on pectic enzymes in general and to discuss in detail what has recently been the subject of a new discovery, viz., pectin *trans*-eliminase.

Until 1960, the well described pectinolytic enzymes produced by micro-organisms were all thought to be hydrolytic in nature.² The enzyme polygalacturonase (PG) facilitates the hydrolysis of $\alpha(1-4)$ glycosidic linkages in the polygalacturonic acid skeleton of pectic or pectinic acids with the resulting formation of polygalacturonic acids of small molecular sizes and of monogalacturonic acid. Pectic acid depolymerase (DP) differs from PG in having a sharp pH optimum at 4.5 and to get activated

by sodium chloride. Pectin methyl esterase (PME) catalyses the cleavage of methyl ester groups in pectinic acids and pectins to yield pectic acids and methanol. The mode of action of PG and PME on macromolecular pectin is illustrated in Fig. 2.

ACTION OF METHYLESTERASE



ACTION OF POLYGALACTURONASE

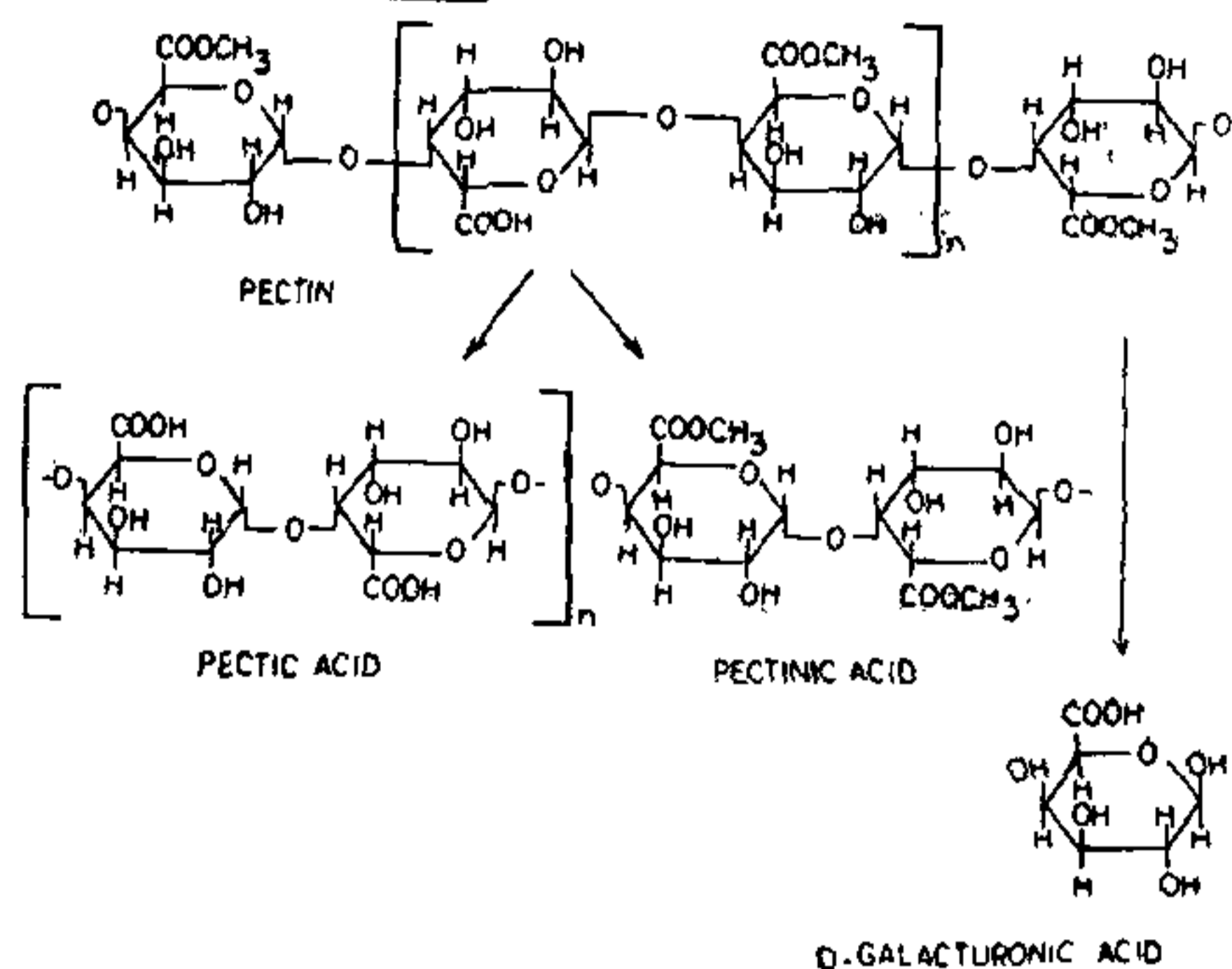


FIG. 2. Action of PG and PME

Investigations on the alkaline degradation of pectin by Neukom and Deuel³ led to the discovery of the new pectinolytic enzyme pectin *trans*-eliminase. The mechanism of this degradation was explained on the lines of " β -dealkoxylation" according to which, the removal of activated hydrogen at C-5 resulted in the formation of a double bond between C₄ and C₅ and the cleavage in β -position to the ester carbonyl group. Subsequently, Albersheim, Neukom and Deuel⁴ observed that such a '*trans*-elimination' also took place on heating pectin in a buffer solution at pH 6.8. The products of degradation showed an absorption maximum at 230-235 m μ , reacted with thiobarbituric acid to give a coloured product having an absorption maximum at 547 m μ and formed oxalic acid with ozone. These characteristics provided additional evidence for a *trans*-elimination reaction consequent to the removal of H atom at C-5, formation of a double bond between C-4 and C-5, and elimination of glycosidic residue at C-4. Later, Albersheim, Neukom and Deuel⁵ demonstrated the presence, in commercial

pectinase preparation, "Pectasin-R-10", of an enzyme that brought about such a *trans*-elimination reaction and designated it as pectin *trans*-eliminase. The purification of this enzyme was achieved by Albersheim and Killias⁶; they also described its properties. Subsequently, Albersheim was able to obtain it in a crystalline form.⁷ According to the substrate specificity exhibited, these enzymes have been now designated pectin *trans*-eliminase (PTE) or polygalacturonic acid *trans*-eliminase (PATE) (Fig. 3). The product of this reaction has been fully characterised by Hasegawa and Nagel⁸ as 4-O α -D-(4, 5-dehydrogalacturonosyl)-D-galacturonic acid (Fig. 4).

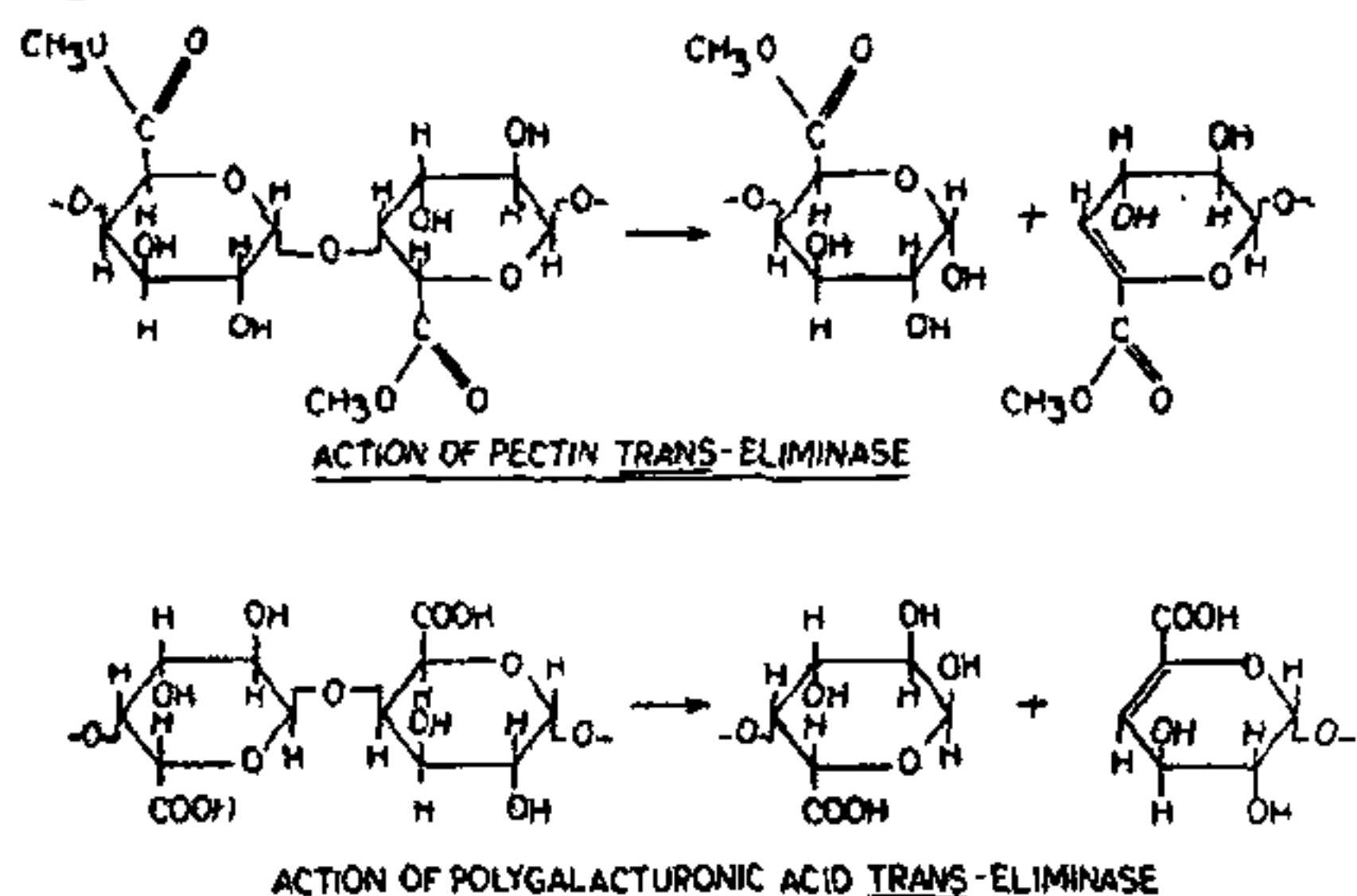


FIG. 3. *Trans*-Eliminase Enzymes

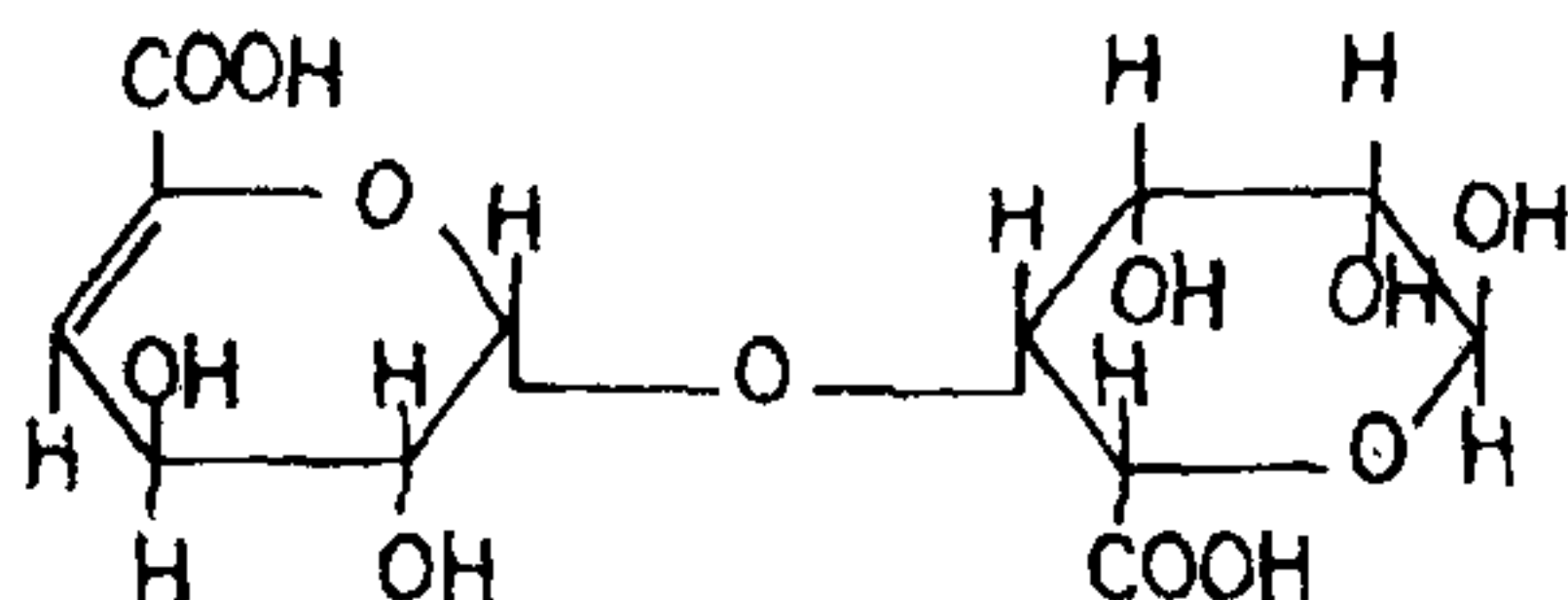


FIG. 4. Structure of Dimer

It has now become apparent that a number of microbes may be producing *trans*-eliminases and it is reasonable to assume that many of the polygalacturonases described prior to 1960 (before the discovery of PTE by the Swiss group of workers) may have to be recharacterized.⁹ The polymethyl galacturonase from commercial pectinase—"Hydrolase"—described by Seegmiller and Jansen¹⁰ and pectin depolymerase from *Neurospora crassa* described by Roboz, Barratt and Tatum¹¹ are cases in point.

Since the discovery of the enzyme *trans*-eliminase, the principal mechanism involved in bacterial degradation has been shown to be due to the *trans*-eliminative cleavage of glycosidic linkages. While investigating the pectic enzymes of *Bacillus polymyxa*, Nagel¹² observed an altered digalacturonic acid differing from the normal compound. The factors affecting growth and enzyme secretion by this organism

have since been studied elaborately by Nagel and Vaughn¹³⁻¹⁵ and the *trans*-eliminative mechanism was shown to occur in bacterial degradation of pectic substances. Starr and Moran,^{16,17} working on the enzymes of the phytopathogenic soft rot bacteria as *Erwinia* and *Bacillus*, recorded results substantiating these findings. Starr and Nasuno¹⁸ subsequently indicated the presence of an extracellular enzyme in *Xanthomonas* sp. which has a specific action on polygalacturonic acid rather than pectin.

Recent work carried out in this laboratory on the culture filtrates of some bacteria characterized as *Xanthomonas*, the enzyme PTE/PATE was not detected.^{19,20} However, data obtained seem to suggest that these cultures, in addition to PG, possess a hitherto unrecognized mode of action on pectic substances, since all the breakdown products could not quantitatively account for those that might have resulted from the action of PG alone. The enzyme PME was not detected in their system. On the other hand, the enzyme *trans*-eliminase has been demonstrated in bacterial cultures of *Corynebacterium barkeri*,²¹ *Flavobacterium* sp.,²¹ *Micrococcus* sp.,^{22,23} and *Arthrobacter* sp.²⁴ Preiss and Ashwell²⁵ reported the presence of this enzyme in *Pseudomonas* sp. and Hsu and Vaughn²⁶ in *Aeromonas liquefaciens*. Recently, Nagel and Anderson²⁷ studied the action of the enzymes of *Bacillus polymyxa* on normal and unsaturated oligogalacturonic acids while Dave and Vaughn²⁸ gave evidence to indicate that, in contrast to other polygalacturonic acid *trans*-eliminases studied, the PATE of *Bacillus pumilus* produced a large quantity of unsaturated trigalacturonic acid.

MacMillan and Vaughn²⁹ have discovered yet another bacterial polygalacturonic acid *trans*-eliminase in a strain of *Clostridium multifementans*; the enzyme attacked the terminal groups to produce a preponderance of unsaturated digalacturonic acid. The organism was also reported to produce the demethylating enzyme so that, grown *in vitro* or in nature, the cultures can bring about the degradation of pectin as far as the dimer stage. However, it appears to be unable to produce the digalacturonase; in other words, it is unlike *B. polymyxa*¹² in so far as it does not destroy completely the pectic material, though it may share several other properties with it. Such a terminally acting PATE was also observed in certain *Erwinia* sp. by Okamoto and Ozawa.³⁰

The presence of the enzyme PTE in different species of the mould *Aspergillus* was demonstrated by Edstrom and Phaff.³¹ These authors purified the enzyme PTE from the culture fluid of *Aspergillus fonsecæus* and demonstrated that the enzyme was specific for pectin and differed in several respects from a PATE produced by certain bacteria. The enzyme had an optimum pH at 5.2 and was not absolutely dependent on Ca ions unlike the bacterial *trans*-eliminases. These authors further provided the course of action by *A. fonsecæus* PTE on pectin and on certain oligogalacturonide methyl esters.³²

That the actinomycetes could be considered as a potent group for the production of pectinolytic enzymes have been proved by the successful investigations in this laboratory.^{33,34} Simultaneously Kaiser³⁵ reported the isolation of pectinolytic actinomycetes by exploitation of the method developed by Wieringa.³⁶ It was demonstrated by Agate²¹ and Agate, Bilimoria and Bhat³⁷ that several species of *Streptomyces* possessed *trans*-eliminase activity. The streptomycetal *trans*-eliminase in general resembled the bacterial enzymes, but differed from the fungal *trans*-eliminase.

A PTE (and PATE) was detected in crude acetone powder prepared from protozoa occurring in the rets of *Calotropis* and *Hibiscus* plant straws.³⁸ Subsequently, it was demonstrated conclusively in the protozoan species *Plagiopyla*.²¹ A PATE, unlike the PTE in *S. viridochromogenes* was demonstrated in an *Epistylis* sp. occurring in sewage and sewage products.³⁹ Later on, two other protozoal species from the same ecosystem, viz., *Vorticella*, *Carchesium* were found to contain this enzyme²¹ and the inclusion of protozoa in the already existing list of micro-organisms producing PTE was a contribution in the field from this laboratory.

Although these interesting *trans*-eliminases have been found to be produced by bacteria, actinomycetes, moulds and even protozoa, to date in our laboratory we have not been able to detect this enzyme in the large collection of pectinolytic yeasts that we have, including such pectinolytic species as *Cryptococcus laurentii*, *Saccharomyces kluyveri*, *Cryptococcus diffluens*, *Rhodotorula glutinis*, *R. flava*, etc.^{19,21,24} This finds confirmation in the fact that Vaughn also could not detect the presence of any *trans*-eliminase enzyme in the pectinolytic yeasts, e.g., in *Saccharomyces fragilis* or *Rhodotorula glutinis*.⁹

The discovery of this enzyme has broken new grounds in the field of microbial pectinolytic

enzymes and it is hoped that a better understanding of these enzymes would help to bring a little order into the chaos prevailing in the field of pectin chemistry.

1. Vanquelin, M., *Ann. Chim.* 1790, 5, 92.
2. Demain, A. L. and Phaff, H. J., *Wallerstein Lab. Commn.*, 1957, 20, 110.
3. Neukom, H. and Deuel, H., *Chem. Ind.*, 1958, p. 683.
4. Albersheim, P., Neukom, H. and Deuel, H., *Arch. Biochem. Biophys.*, 1960, 90, 46.
5. —, — and —, *Helv. Chim. Acta*, 1960, 43, 1422.
6. — and Killias, U., *Arch. Biochem. Biophys.*, 1962, 97, 107.
7. —, *Plant Physiol.*, Supplement XXXIX, 1961.
8. Hasegawa, S. and Nagel, C. W., *J. Biol. Chem.*, 1962, 237, 619.
9. Vaughn, R. H., *Microbiological Quality of Foods*, Academic Press, New York, 1963.
10. Seegmiller, C. G. and Jansen, E. F., *J. Biol. Chem.*, 1952, 195, 327.
11. Roboz, E., Barratt, R. W. and Tatum, E. L., *Ibid.*, 1952, 195, 459.
12. Nagel, C. W., *Ph.D. Dissertation*, University of California, Davis, 1960.
13. — and Vaughn, R. H., *Arch. Biochem. Biophys.*, 1961, 93, 344.
14. — and —, *Ibid.*, 1961, 94, 328.
15. — and —, *J. Bacteriol.*, 1962, 83, 1.
16. Starr, M. P. and Moran, F., *Bacteriol. Proc.*, 1961, p. 169.
17. — and —, *Science*, 1962, 135, 920.
18. — and Nasuno, S., *Bacteriol. Proc. Abstr.*, 1963, p. 116.
19. Bilimoria, M. H., *Ph.D. Thesis*, Indian Institute of Science, Bangalore, 1962.
20. —, *J. Indian Inst. Sci.*, 1966, 48, 53.
21. Agate, A. D., *Ph.D. Thesis*, Indian Institute of Science, Bangalore, 1965.
22. Jayasankar, N. P. and Bhat, J. V., *Curr. Sci.*, 1964, 33, 369.
23. —, Agate, A. D. and Bhat, J. V., *J. Ind. Inst. Sci.*, (In press).
24. —, *Ph.D. Thesis*, Under preparation.
25. Preiss, J. and Ashwell, G., *J. Biol. Chem.*, 1963, 238, 1571.
26. Hsu, E. J. and Vaughn, R. H., *Bacteriol. Proc.*, 1965, p. 11.
27. Nagel, C. W. and Anderson, M. M., *Arch. Biochem. Biophys.*, 1965, 112, 322.
28. Dave, B. A. and Vaughn, R. H., *Bacteriol. Proc.*, 1966, p. 6.
29. MacMillan, J. D. and Vaughn, R. H., *Biochemistry*, 1964, 3, 564.
30. Okamoto, K. and Ozawa, J., *Nôgaku Kenkyû*, 1960, 48, 39.
31. Edstrom, R. D. and Phaff, H. J., *J. Biol. Chem.*, 1964, 239, 2403.
32. — and —, *Ibid.*, 1964, 239, 2409.
33. Bilimoria, M. H., *Chemical Process. Design Symposium*, C.S.I.R., India, 1963.
34. — and Bhat, J. V., *Indian Patent*, 1963, 75, 325.
35. Kaiser, P., *D.Sc. Thesis*, Paris University, 1961.
36. Wieringa, K. T., *Proc. IV Int. Cong. Microbiol.*, Copenhagen.
37. Agate, A. D., Bilimoria, M. H. and Bhat, J. V., *Curr. Sci.*, 1962, 31, 402.
38. — and Bhat, J. V., *J. Indian Inst. Sci.*, 1963, 45, 49.
39. — and —, *Curr. Sci.*, 1963, 32, 459.