dye is stated to be 87·3% pure and we have found that a mixture containing 0·75 ml. of 0·1% dye solution for every 5 ml. of the 0·5% bruciquinone indicator solution gives a sharp colour change from deep pink to green. One drop of this mixture is found to be sufficient for every 40 ml. of the titration mixture in contrast to 2 drops required when bruciquinone alone is employed as indicator.

Department of Chemistry, T. P. SASTRY.
Regional Engineering College, P. S. SASTRY.
P.O. Kazipet, *July* 27, 1965. M. RAMAIAH.

1. Leuchs, H., Seeger and Jaegers K., *Ber.*, 1938, **71** 2023.
2. Sastry, T. P. and Samuel Pratt III, *Z. anal. Chem.*, 1960, **175**, 182.

## TETRANITROAZOXYBENZENE AND PHOTOINDUCED CONDENSATIONS OF POLYNITROAROMATICS

STENBERG AND HOLTER[1] recently have reported the formation of 3, 5, 3', 5'-tetranitroazoxybenzene (I) by irradiation of 1, 3, 5-trinitrobenzene in ethanol and tetrahydrofuran. They have stated that photo-chemical condensations of polynitroaromatic compounds have not been reported earlier and that (I) was prepared with difficulty by a non-photochemical method.[2]

Photochemical decomposition of polynitroaromatic compounds leading to the formation of azoxy derivatives through intermediate nitroso compounds has already been reported.[3,4] The formation of a dicarboxy derivative of 3, 5, 3', 5'-tetranitroazoxybenzene (II) itself has also been reported by the present authors,[5] by the photochemical condensation of 2, 4, 6-trinitrobenzaldehyde with an intermediate formation of 2, 4-dinitro-6-nitrosobenzoic acid. The identity of 2, 2'-dicarboxy-3, 5, 3', 5'-tetranitroazoxybenzene with the "white compound" formed as a by-product in a continuous TNT manufacture has also been reported. 3, 5, 3', 5'-tetranitroazoxybenzene can be obtained without any difficulty in a good yield by the procedure described below:

10 g. of (II) was refluxed with 100 ml. of pyridine on a sand-bath for 1·5 hours. The black mixture was poured in water and acidified with dil. $H_2SO_4$. The black residue was filtered and washed free of acid. After washing, the residue was dried and extracted with boiling toluene with addition of little animal charcoal. The toluene extract on cooling gave pink-coloured needles of (I). Final crystallisation was from acetic acid.

Yield—5 g., m.p. 190°.

Anal. calcd. for $C_{12}H_6N_8O_9$: Mol. wt., **378**, N, 22·22.

Found: Mol. wt., 376, N, 22·26.

Explosives Research and Development Laboratory, Kirkee, Poona-3, *July* 3, 1965. W. D. PATWARDHAN. S. A. JOSHI.

1. Stenberg, V. I. and Holter, D. J., *J. Org. Chem.*, 1964, **29**, 3420.
2. Lobry De Bruyn and van Leent, A., *Rec. Trav. Chim.*, 1894, **13**, 148.
3. Bamberger and Elger, *Ann.*, 1910, **371**, 319.
4. Patwardhan, W. D., *Defence Sci. Jour. India*, 1954, **4**, 67.
5. Joshi, S. A. and Patwardhan, W. D., *Curr. Sci.*, 1953, **22**, 239.

## PROTEOLYTIC ACTIVITY OF SOME PLANT LATEX
### (Effect of Time Variation)

IN a previous communication[1] we reported the proteolytic activity of fourteen samples of latex collected from different families and species of plants. The pH optima for five of these latices available in appreciable quantities were also determined by employing different buffers at various pH. As a result it was observed that the latices of *Calotropis gigantea* and *Carica papaya* have 6 as their pH optima. In addition, it was found that the latices of *Calotropis procera* and *Cryptostegia grandiflora* exhibited two distinct peaks of activity each at pH 4 & 8 and 5 & 7 respectively. The existence of these two peaks at different pH led us to suggest the presence of two components possessing proteinase activity. The foregoing describes the effect of time variation on the proteolytic activity of five samples tested previously at pH 7.

*Materials.*—Fresh latex was collected from different plants having healthy vigorous growth. Leaves and tender shoots were nipped and the oozing milky sap was allowed to drain in clean dry tubes kept immersed in salt-ice mixture. In cases where yield of latex was scanty, collection was made from more than one plant growing in the neighbourhood and pooled. The best time for collection was found to be early morning before sunrise when the plants were quite turgid and afforded the maximum yield of latex.

*Determination of Proteolytic Activity.*—Proteolytic activity was measured as described by Yamafuji and Yoshihara.[2] The amount of tyrosine released was estimated by the method of Lang and Wegner[3] as described by Turba.[4]

A known volume (0·5 ml.) of latex was diluted to 2 ml. with sodium carbonate (0·2 N)

followed by the addition of 2 ml. each of casein solution (2%, w/v, prepared as described by Balls and Lineweaver[5]) and phosphate buffer at pH 7. The mixture was well stirred and incubated at 37–38° for the desired length of time. After incubation the reaction was stopped by the addition of trichloroacetic acid (4 ml., 0·25 m), allowed to stand for 10 minutes at room temperature and then spun. The precipitated undigested protein was discarded and proteolytic activity was determined in the clear supernatant.

0·1–1·0 ml. of the supernatant was diluted to 2 ml. with distilled water and α-nitroso-β-naphthol in ethanol (0·05 ml., 1 mg.%, w/v) was added to it followed by a solution of ferric ammonium sulphate (2 ml., prepared by mixing 5 parts of saturated solution of ferric ammonium sulphate and 1 part of concentrated nitric acid, sp. gr. 1·42). The mixture was carefully brought to boiling and allowed to stand for one hour at room temperature. The colour developed was read in a Bausch and Lomb Spectronic 20 colorimeter at 400 mμ against a blank identically treated from the supernatant of the control. The amount of tyrosine released was calculated from a calibration curve prepared earlier by measuring the optical density of standard tyrosine at different concentrations. The proteolytic activity was expressed in terms of mg. of tyrosine liberated by 100 mg. of latex at pH 7.

The results obtained as represented in Fig. 1 evince that of the five latices tested, the latex

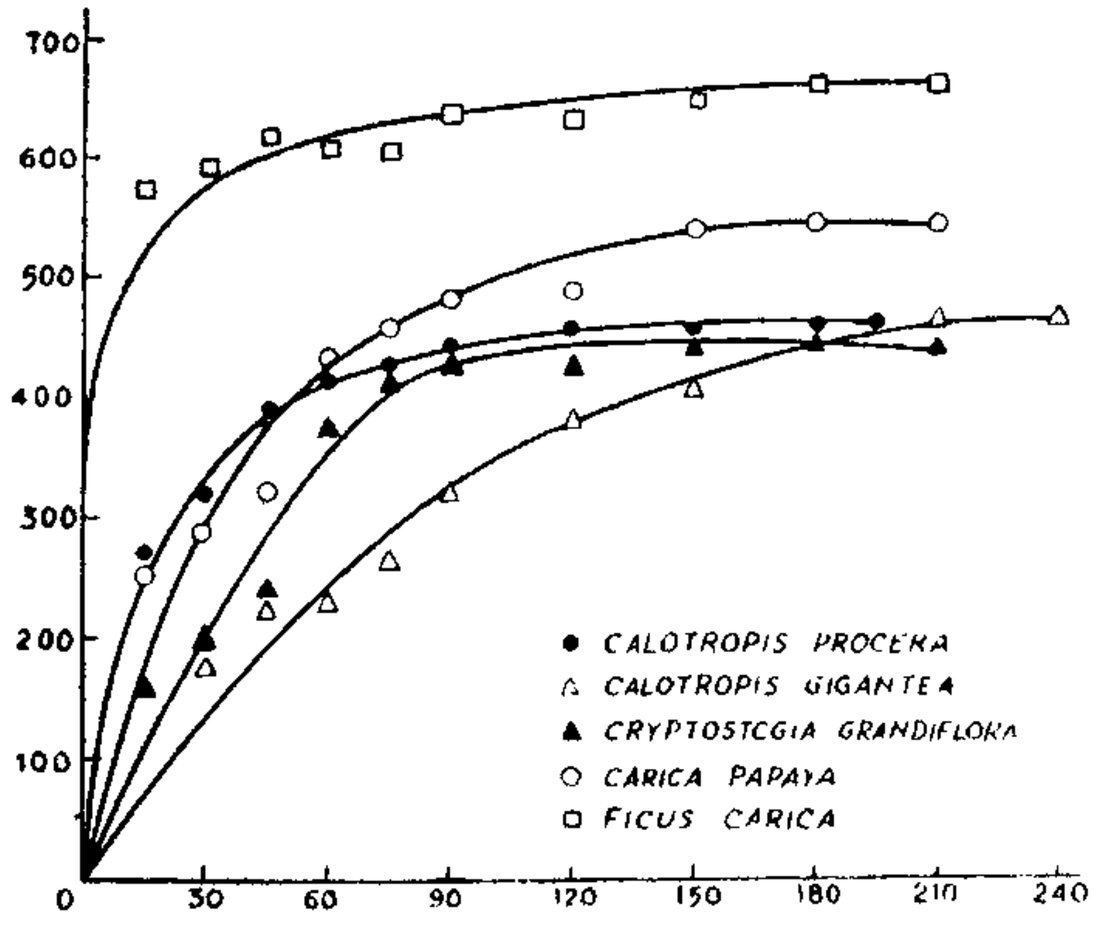


FIG. 1. Effect of time variation on the proteolytic activity of plant latices.

of *Ficus carica* possesses the maximum activity, the optimum being 45 minutes. In the case of *Calotropis procera, Cryptostegia grandiflora, Carica papaya* and *Calotropis gigantea* the optimum time observed was 75, 120, 150 and 210 minutes respectively.

Our thanks are due to the Council of Scientific and Industrial Research, New Delhi, India, for financial assistance and a research assistantship to one of us (S. C. S.).

Biochemistry Section, RADHA PANT.
The University, S. C. SRIVASTAVA.
Allahabad, *July* 27, 1965.

1. Pant, Radha and Srivastava, Subhash, C., *Hoppe Seyler Z.*, 1964, **338**, 32.
2. Yamafuji, K. and Yoshihara, F., *Enzymologia*, 1960, **22**, 157.
3. Lang, K. and Wegner, E., *Biochem. Z.*, 1948, **318**, 462.
4. Turba, F., In *Hoppe-Seyler Thierfelder, Handb. d-Physiol.—und pathol—chem. Analyse*, Springer. Verlag, Heidelberg, Bd. III/**2**, 1955, p. 1742.
5. Balls, A. K. and Lineweaver, H., *J. biol. Chem.*, 1939, **130**, 669.

## IDENTIFICATION OF MUSTARD OIL AND CASTOR OIL IN OTHER OILS BY PAPER CHROMATOGRAPHY

IN examining edible oils for adulteration, the analyst is sometimes called upon to identify the constituents of a mixture of oils. Specific identification tests are available only for sesame oil and cottonseed oil. The presence of other oils can only be inferred from a collation of analytical data such as the refractive index, iodine value, Bellier test, etc. Mustard oil and castor oil differ from other oils in that they contain the unsaturated fatty acids, erucic acid and ricinoleic acid respectively. We have found that separation of these acids by reverse phase chromatography and their identification with iodine offers an easy and reliable method of identifying mustard oil and castor oil when present to the extent of 5% or more in other oils. The method we have used is briefly reported below.

The mixed fatty acids are prepared as in the well-known titre test. Strips of Whatman No. 4 paper, 25 cm. × 25 cm., are prepared by dipping them through a solution of 10% liquid paraffin in ether and drying in air. About 50 microgram of the mixed fatty acids in the form of a chloroform solution is spotted. We have used the ascending technique. After trials with various solvent combinations, the following solvent, not so far reported, was found to be well suited for routine analysis: Acetic acid 6, water 2, amyl acetate 2, saturated with medicinal liquid paraffin. Satisfactory separation results in about 16 hours. The developed chromatogram is air-dried and the unsaturated fatty acids are located by exposing the paper, rolled