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Note

Chromogenic reagent for vicine and convicine on thin-layer plates

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The aglycones of vicine and convicine have been implicated as the causative factors for favism in fababeans[1–4]. Separation of vicine and convicine by thin-layer chromatography (TLC) on cellulose plates was reported by Jamalian *et al.*[4] and Olsen and Andersen[5] who identified these compounds under UV light. This paper reports a new chromogenic reagent (2% titanium tetrachloride in conc. hydrochloric acid) for detecting vicine and convicine on cellulose coated sheets (Eastman-Kodak) based on the complex formation of the aglycones with titanium[6]. In order for the complex to form the aglycone must be produced by acid treatment of the glycosides (Fig. 1). This is required to release the hydroxyl group for interaction with the titanium salt. The titanium reagent has been developed in our laboratory to measure hydrogen peroxide[7],

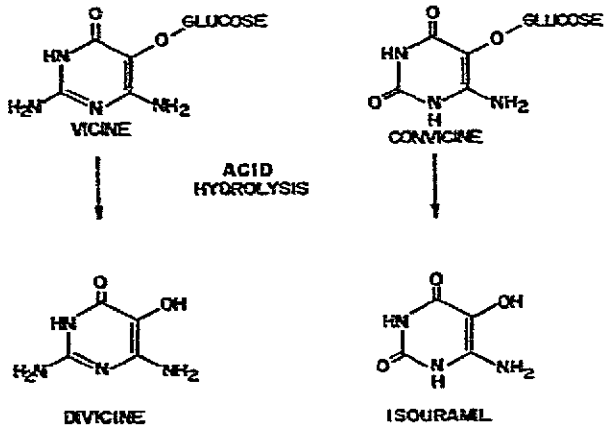

Fig. 1. Acid hydrolysis of vicine and convicine to their respective aglycones, divicine and isouramil.

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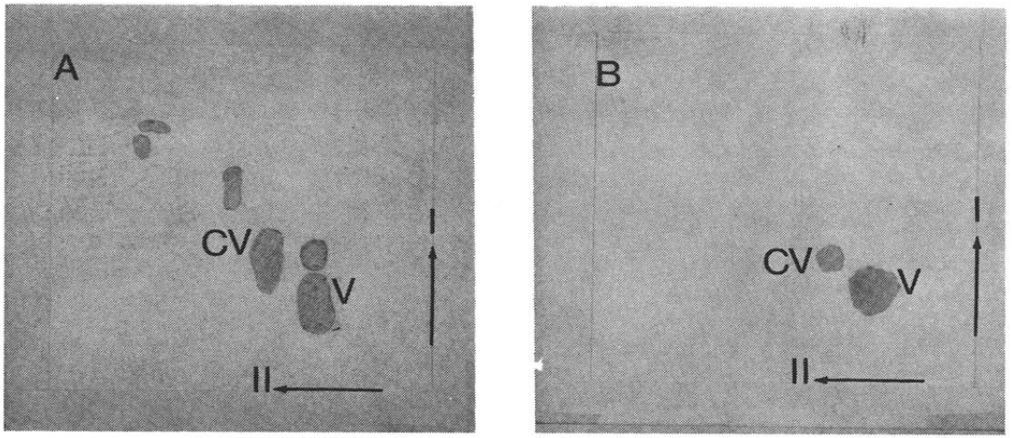

Fig. 2. Separation of vicine and convicine in fababean extracts by two-dimensional TLC. Dimension I: solvent 1, ethanol–water (95:5); dried then developed with solvent 2, methanol–25% ammonia–water (14:1:5); dimension II: solvent 3, methanol–0.02 *M* phosphate buffer pH 5.8 (7:3). A, 2% trichloroacetic acid extract; B, extract following aluminum oxide treatment. Abbreviations, V, vicine and CV, convicine.

organic hydroperoxides[8], phenolic compounds[9] and sinapine[10] as well as a chromogenic reagent for phenolics on thin-layer plates[11].

Pure vicine and convicine solutions ranging in concentration from 0.1–20 μg/2 μl were applied to cellulose plates and developed by TLC according to the procedure outlined by Olsen and Andersen[5]. The plates were air dried, sprayed with conc. hydrochloric acid and heated over a hot tray for 2 min to produce the aglycones followed by spraying with the titanium reagent. In both cases the limit of detection by the titanium reagent was 1 μg resulting in a brown spot of approximately 0.4 cm^2 area. The applicability of the reagent to detect vicine and convicine in 2% trichloroacetic acid extracts of fababean was examined before and after treatment with aluminum oxide by two-dimensional TLC. The chromatograms shown in Fig. 2 indicate that vicine and convicine could be readily detected in the fababean extracts. It is evident that interference by phenolic compounds can be eliminated by aluminum oxide treatment of the extract. No additional spots were evident in the fababean extract using the titanium reagent although additional ones were reported by Olsen and Andersen[5] using UV light as detector. Related pyrimidine and purine compounds did not react with the titanium reagent although they are known to respond to UV light. The titanium–aglycone spots were stable for several months. It is evident that the titanium reagent provides a simple and sensitive method for identifying vicine and convicine via their aglycones on thin-layer plates.

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REFERENCES

1 J. Y. Ling and K. H. Ling, *J. Formosan Med. Assoc.*, 61 (1962a) 490.
2 J. Y. Ling, *J. Formosan Med. Assoc.*, 61 (1962b) 579.
3 J. Mager, G. Glaser, A. Razin, G. Izak, S. Bien and M. Noam, *Biochem. Biophys. Res. Commun.*, 20 (1965) 235.
4 J. Jamalian, F. A. Aylward and J. F. Hudson, *Qual. Plant Foods Hum. Nutr.*, 27 (1977) 213.
5 H. S. Olsen and J. H. Andersen, *J. Sci. Food Agr.*, 29 (1978) 323.
6 E. Hoehn, N. A. M. Eskin, S. I. Kim and F. Ismail, *J. Agr. Food Chem.*, in preparation.
7 C. B. Gupta, N. A. M. Eskin, C. Frenkel and A. Y. Sadovski, *J. Food Sci.*, 42 (1977) 537.
8 N. A. M. Eskin and C. Frenkel, *J. Amer. Oil Chem. Soc.*, 53 (1976) 74.
9 N. A. M. Eskin, E. Hoehn and C. Frenkel, *J. Agr. Food Chem.*, 26 (1978) 973.
10 F. Ismail and N. A. M. Eskin, *J. Agr. Food Chem.*, 27 (1979) 917.
11 N. A. M. Eskin and C. Frenkel, *J. Chromatogr.*, 150 (1978) 293.