ISOLATION OF INDOLE-3-ALDEHYDE FROM *PSEUDOMONAS SYRINGAE* PV. *SAVASTANOI*

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Pseudomonas syringae pv. *savastanoi* is the causal agent of olive and oleander knot disease. Extensive studies on olive and oleander plants have shown that plant growth substances, indole-3-acetic acid (IAA) and cytokinins, of bacterial origin are involved in the production of disease symptoms (1,2). Pathovar *savastanoi* also possesses several systems that affect accumulation of IAA in culture. These include the conversion of IAA to lysine conjugates (3,4) possessing less growth stimulating activity. We now report the isolation and identification by means of ^{1}H nmr and ms of another product of IAA metabolism in pv. *savastanoi*.

An accurate tlc analysis (SiO_2, $CHCl_3$-EtOAc-MeOH, 2:2:1) of the organic extracts of the acidified culture filtrate of the olive strain ITM[1] 317 showed the presence of IAA and another indole compound chromatographically different from the indoles previously detected in the pv. *savastanoi* culture filtrates.

On the basis of its chemical and physical properties (uv, ir, ^{1}H nmr, and ms), the structure of indole-3-aldehyde has been assigned to the unknown indole derivative.

The indole-3-aldehyde is a known product of the degradative metabolism of IAA. In particular, it is considered as an alternate end-product of the oxidation of IAA by horseradish peroxidase with indolenine hydroperoxide and indolenine epoxide as intermediates (5). The same pattern of reactions could occur in pv. *savastanoi* since a high peroxidase activity has been observed in pv. *savastanoi* enzyme preparations (6). Moreover, indole-3-aldehyde accumulated in 5-day-old cultures of strain ITM 317 but not in the cultures of its IAA deficient mutant (IAA nonproducer).

Indole-3-aldehyde is a stable derivative of IAA naturally occurring in some plants and can also be obtained after oxidation of IAA by plant enzyme preparation (7). To our knowledge, this represents the first isolation of indole-3-aldehyde from culture filtrate of a bacterium whose hormonal production (IAA and/or cytokinin) has a decisive effect on its ability to be a pathogen (i.e., forms knots).

EXPERIMENTAL

BACTERIAL STRAIN.—*P. syringae* pv. *savastanoi* strain ITM 317 was isolated in 1982 from olive knots and deposited in the bacteria collection of the Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, Bari, Italy.

PRODUCTION AND FRACTIONATION OF INDOLES.—Pathovar *savastanoi* ITM 317 was grown in 400 ml of Woolley's medium (8) using 1 liter Erlenmeyer flasks on a rotary shaker for 5 days at 26°. The culture filtrates (6.7 liters) were lyophilized, resuspended in distilled H_2O (650 ml), acidified to pH~2 with 1N HCl and extracted with EtOAc (4×300 ml). The oil residue (93.2 mg), obtained by EtOAc evaporation under reduced pressure, was chromatographed on SiO_2 plates (eluent $CHCl_3$-EtOAC-MeOH, 2:2:1) yielding two uv absorbing bands which were scraped off and eluted with MeOH. Evaporation of the solvent gave pure IAA (27 mg) and indole-3-aldehyde in a mixture. A further purification by preparative tlc on SiO_2 (eluted with the same solvent system) gave the indole-3-aldehyde as an oil (5.4 mg) which resisted crystallization. The uv and the ir and ms spectra were consistent with the data reported in the literature (9) and (10), respectively; ^{1}H nmr (270 MHz, CD_3OD) δ 9.88 (s, H-10), 8.16 (dd, J=8.0, 1.5 Hz, H-4), 8.09 (s, H-2), 7.48 (dd, J=8.0, 1.5 Hz, H-7), 7.28 (ddd, J=8.0, 8.0, 1.5 Hz, H-6), 7.23 (ddd, J=8.0, 8.0, 1.5 Hz, H-5).

Full details of the isolation and identification of the compound are available on request to the authors.

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[1]ITM, Collection of Istituto Tossine e Micotossine da Parassiti Vegetali del CNR, Bari, Italy.

LITERATURE CITED

1. M. Smidt and T. Kosuge, *Physiol. Pl. Pathol.*, **13**, 203 (1978).
2. G. Surico, N.S. Iacobellis and A. Sisto, *Physiol. Pl. Pathol.*, **26**, 309 (1985).
3. O. Hutzinger and T. Kosuge, *Biochemistry*, **7**, 601 (1968).
4. A. Evidente, G. Surico, N.S. Iacobellis and G. Randazzo, *Phytochemistry*, **25**, 125 (1986).
5. R.L. Hinman and J. Lang, *Biochemistry*, **4**, 144 (1965)
6. A.R. Magie, E.E. Wilson and T. Kosuge, *Science*, **141**, 1281 (1963).
7. E.A. Schneider and F. Wightman, Auxins, in: "Phytohormones and Related Compounds—A Comprensive Treatise", Ed. by D.S. Letham, P.B. Goodwin, and T.J.V. Higgins, Vol. 1, Elsevier/North-Holland Biomedical Press, New York, 1978, pp. 65-66.
8. D.W. Woolley, G. Schaffner, and A.C. Brown, *J. Biol. Chem.*, **215**, 485 (1955).
9. A.I. Scott, in: "Interpretation of the Ultraviolet Spectra of Natural Products," Ed. by D.H.R. Barton and W. Doering, Vol. 7. Pergamon Press, Oxford, 1964, p. 176.
10. B.K. Chowdhury and D.P. Chakraborty, *Phytochemistry*, **10**, 481 (1971).

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CYCLOPEPTIDE ALKALOIDS FROM *ZIZYPHUS XYLOPYRA*

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In our continuing search (1-7) for cyclopeptide alkaloids from *Ziziyphus* species, we report here the isolation and characterization of the cyclopeptide alkaloids mauritine-D and nummularine-B from the bark of *Zizyphus xylopyra* Willd. These alkaloids have not previously been reported from this source.

EXPERIMENTAL

PLANT MATERIAL.—The bark of *Z. xylopyra* used in this investigation is collected from Varanasi district, UP, India. A voucher specimen is kept in the Department of Medicinal Chemistry, Banaras Hindu University.

EXTRACTION AND ISOLATION OF PEPTIDE ALKALOIDS.—Air-dried, finely powdered bark (4 kg) of *Z. xylopyra* was exhaustively extracted with a mixture of C_6H_6-MeOH-NH_4OH (100:1:1), and the crude alkaloids (0.4 g) were isolated in the usual manner (8). The crude alkaloids were fractionated on a silica gel column, eluting with increasingly polar $CHCl_3$/MeOH mixtures. The cyclopeptide alkaloids, mauritine-D (8 mg) (9) and nummularine-B (7 mg) (10), were obtained by repeated preparative tlc of the appropriate fractions eluted from the above column and identified by ir, uv, ^{1}H nmr, ms, and hydrolysis.

The structures were confirmed by comparison with authentic samples (mp, mmp, co-tlc, and superimposable ir).

Full details of the isolation and identification of the compounds are available on request to the senior author.

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LITERATURE CITED

1. R. Tschesche, A.H. Shah, V.B. Pandey, J.P. Singh, M.V. Radloff, and G. Eckhardt, *Pharmazie*, **36**, H7 (1981).
2. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, *Phytochemistry*, **24**, 2765, (1985).
3. A.H. Shah, V.B. Pandey, G. Eckhardt, and R Tschesche, *Phytochemistry*, **24**, 2768 (1985).
4. A.H. Shah, V.B. Pandey, G. Eckhardt, and R. Tschesche, *Phytochemistry*, **23**, 931 (1984).

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