Phytochemistry, 1977, Vol. 16, pp. 1296–1297. Pergamon Press. Printed in England.

4-ACETAMIDO-2-BUTENOIC ACID FROM *FUSARIUM GRAMINEARUM*

RONALD F. VESONDER, LARRY W. TJARKS, ALEX CIEGLER, GAYLAND F. SPENCER and LOWELL L. WALLEN

Northern Regional Research Centre, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604, U.S.A.

(*Revised received* 14 *January* 1977)

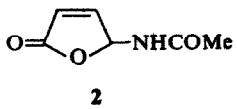
Key Word Index—*Fusarium graminearum*; fungi; secondary amide; acetamide acid.

The etiology of many feeding problems of farm animals, especially swine, has been associated with toxins found in corn contaminated naturally with *Fusarium*. Recently, we showed the trichothecene 3,7,15-trihydroxy-12,13-epoxy-trichothec-9-en-8-one (vomitoxin), isolated from corn naturally contaminated with predominantly *Fusarium graminearum*, to be an emetic to swine [1], as well as the factor responsible for refusal of feed by swine [2].

Fractionation of the extract containing vomitoxin from rice fermented with *F. graminearum* NRRL 5883, the culture obtained from naturally infected corn, resulted in the isolation of a new metabolite. The structure 4-acetamino-2-butenoic acid (**1**) was assigned to this metabolite on the basis of spectroscopic and chemical evidence.

$$MeCOCH_2CH{=}CHCO_2H$$

1



2

Crystallization from MeOH–$CHCl_3$ gave the pure, water-soluble, *N*-acetyl amide **1** (mp 140°) of formula $C_6H_9NO_3$, *m/e*, 143. The IR spectrum indicates non-cyclic secondary amide NH at 3520, 3100, carbonyl absorption 1712 and carbonyl of secondary amide 1630, 1570 cm^{-1}, from films deposited on KRS-5 plate from acetone solution. The signal for the methyl of the acetamido group in the PMR spectrum occurs at δ 1.95 (3H, *s*, $NCOCH_3$) in d_4-MeOH and δ 2.47 in deuterated trifluoroacetic acid. The other protons in the NMR spectrum in d_4-MeOH were in accord with the assigned structure; δ 4.28 (2H, *m*, $-CH_2$), 5.82 (1H, pr. *t*, J = 11 Hz), and 6.16 (1H, pr. *t*, J = 11 Hz). The CMR showed the following chemical shifts for **1** in D_2O with dioxane as internal standard; δ 24.5 ($NCOCH_3$, 41.3 ($-CH_2$), 149.1 (C = C, and 176.9 (CO). The CMR and PMR signals for the methyl of the acetamido group of **1**, compare favourably with those exhibited by butenolide (**2**). The PMR signal for the acetamido methyl group in **2** was at δ 2.02 in $CDCl_3$–CD_3OD. In the CMR spectrum, chemical shifts (ppm) and multiplicity due to CH coupling in **2** which compared with **1** are δ 22.7 (*q*, $NCOCH_3$) and 171.8 (*s*, NCOMe); other shifts observed are δ 82.2 (*d*, CH = CH-CH), 124.3 (*d*, CH = CH), and 151.9 (*d*, CH = CH). The presence of a carboxyl group was shown by conversion of **1** to its methyl ester with diazomethane *m/e*, 157.

The NMR spectrum of methyl ester of **1** in $CDCl_3$ exhibited the following signals which support the assigned structure: δ 1.96 (3H, *s*, $COCH_3$), 3.68 (3H, *s*, $COOCH_3$), 4.30 (2H, *m*, CH_2), 5.81 (1H, pr. *t*, J = 11 Hz), 6.25 (1H, pr. *t*, J = 11 Hz) and 6.45 (1H, *bs*, NH. A coupling of J = 6 Hz between the NH proton and the CH_2 group was observed and on addition of D_2O the NH proton exchanged (δ 6.5 disappeared) and the CH_2 multiplet (δ 4.31) collapsed into a pair of doublets. Irradiation of this pair of doublets collapsed the olefinic protons (δ 5.82, 6.24) region into an AB doublet (J = 11 Hz) and irradiation of the olefinic region collapsed the CH_2 doublets into a singlet. In addition, vicinal coupling (CH=CH-CH_2, J = 6 Hz) and allylic coupling (CH=CH-CH_2, J = 2 Hz) were also observed.

To ascertain if the inference is correct from the NMR data that the CH_2 moiety is not α to the carboxyl group, the methyl ester of **1** was subjected to reductive ozonolysis. Formation of methyl glyoxalate was proven by GC–MS analyses and by direct comparison with an authentic sample, thus fixing the position of the double bond at C-2 and C-3.

Potential antibiotic properties and toxicity towards the chick embryo and mouse were determined by standard methods. No toxicity was noted in chick embryo assay at levels of 1–200 μg/egg, nor were teratogenic effects observed. The *N*-acetyl amide **1** was also nontoxic to mice injected intraperitoneally with 100 mg/kg. No antibiotic activity was observed either to gram positive or gram negative bacteria, to mycobacterium, or to yeasts and molds. The possibility that 4-acetamido-2-butenoic acid may be an intermediate in the biosynthetic pathway of butenolide is suggested by the similarity in structure.

EXPERIMENTAL

Production of and purification of **1**. The acetylamide was produced by growing *F. graminearum* NRRL 5883 as still cultures at 28° on rice for 13 days as previously described [2]. The moulded rice was dried in a forced air oven for 4 hr at 80° and extracted in a blender with BuOH (2 × l/kg); the BuOH-

extracted rice was washed with hexane and dried in vented hood. The dry rice was again extracted in a blender with 40° aq. MeOH (2 × 1 l. kg). The combined extracts of sq. MeOH were concd to a small vol. and them freeze-dried to a brown solid which was extracted with MeOH at room temp. for 1 hr. The MeOH was evaporated, and the residue was redissolved in a mimimum amount of MeOH and added to Me_2CO to ppt. insoluble material. The Me_2CO–MeOH solubles were repeatedly treated in this manner until no further ppt. occurred on Me_2CO addition (3 ×). The MeOH–Me_2CO soluble yellow oil was added to the top of a Si gel column (2 × 30 cm) presaturated with $CHCl_3$. The column was washed with 1 l. of $CHCl_3$ followed by elution of crude vomitoxin with 300 ml of 3% MeOH in $CHCl_3$. Further elution (100 ml) gave 220 mg of crude **1** per kg of rice substrate. Crude **1** was crystallized from 3% CH_3OH in $CHCl_3$ (170 mg, mp 140°). (Found- C, 50.3; H, 6.3; N, 9.98; parent ion *m/e* 143. Calc.: C, 50.34; H, 6.34; N, 9.78; MW 143).

Physical and chemical analyses. Mps are uncorrected. The IR spectra were recorded as films deposited on KRS-5 plate from Me_2CO solutions. NMR spectra were recorded with a Varian HA-100. CMR spectra were recorded with a Brüker-WH 90. The acetylamide **1** was esterified by treatment with ethereal CH_2N_2 for 1 hr at room temp. The resulting methyl ester of **1** (shown by NMR and parent *m/e* 152) was treated with ozone for 30 sec in CH_2Cl_2 and reduced with triphenylphosphite. The reaction mixture was taken to dryness and redissolved in Et_2O. The reduced ozonolysis product was analyzed on a Bendix 2600 GC (column- 2 m × 7 mm glass 3% Silar-5 cp) connected through a single-stage jet-type stainless-steel separator to a Dupont 492-1 mass spectrometer; MS (scan taken at 70.eV) were recorded every 4 sec/decade of the GC peaks. An authentic sample of methyl glyoxalate was used for comparison with the oxidation product and showed the same R_f and MS.

Acknowledgements—We thank Ms. Clara E. Johnson for microanalyses, and A. E. Johnston, Jr., for use of ozonolysis apparatus.

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Phytochemistry, 1977, Vol. 16, pp. 1297–1298. Pergamon Press. Printed in England.

EFFECT OF ETHREL AND CHLOROETHANOL ON PEA DIAMINE OXIDASE ACTIVITY

S. K. SRIVASTAVA, V. PRAKASH and B. I. NAIK
Biochemistry Department, Baroda University, Baroda, India

(*Received* 28 *January* 1977)

Key Word Index—*Pisum sativum*; Leguminosae; pea; diamine oxidase; ethylene; ethrel; chloroethanol; 2,4-D.

Abstract—The activity of cotyledon and embryo diamine oxidase was reduced by feeding ethrel and chloroethanol to the seedlings. The inhibitory effect of 2,4-D on the activity of enzyme in the cotyledon which may be mediated through ethylene was reversed by exposure of seeds to red light.

INTRODUCTION

On feeding 2,4-D to intact pea seedlings, the activity of the cotyledon diamine oxidase was reduced [1]. This effect was mediated through the embryo since removal of the embryo after soaking in the 2,4-D for 14 hr abolished the inhibitory effect, suggesting the elaboration of an inhibitor of diamine oxidase activity or synthesis in the embryo. 2,4-D enhances the synthesis of ethylene in tissues [2, 3] and ethylene may therefore control this enzyme. Experiments were carried out using ethrel (2-chloroethyl phosphonic acid) and ethylene chlorohydrin (2-chloroethanol), substances known to form ethylene *in vivo* [3]. The results of this study are reported in the present communication.

RESULTS AND DISCUSSION

The data reported in Table 1 show that the activity of both cotyledon and embryo enzymes as decreased with increasing concentrations of ethrel when the seeds were germinated after soaking them in ethrel for 14 hr. Enzyme activity tends to return to the normal level after a further period of germination. Similar results were obtained with chloroethanol except that the concentration of chloroethanol required was only 10% of that of ethrel.

We have shown earlier [1] that the inhibitory effect of feeding 2,4-D to pea seedlings on the cotyledon enzyme was abolished if the embryo was removed from the seeds after soaking. However, with ethrel and chloroethanol the cotyledon enzyme was inhibited irrespective of whether the embryo was present or not after soaking since the ethylene produced would be directly available in the cotyledon.

Studies were also carried out to investigate whether chloroethanol would affect the enzyme when added 14, 38 and 62 hr after germination. In all the groups the enzyme activity was determined 48hr after adding chloroethanol. The data reported in Table 2 show that both cotyledon and embryo enzymes were inhibited. The inhibitory effect was maximum in the seeds when chloroethanol was added immediately after soaking and