REFERENCES

1 Nes, W R and McKean, M L (1977) *Biochemistry of Steroids and Other Isopentenoids* University Park Press, Baltimore
2 Nes, W R, Krevitz, K, Joseph, J, Nes, W D, Harris, B, Gibbons, G F and Patterson, G W (1977) *Lipids* **12**, 511
3 Hanna, R (1964) *Lloydia* **27**, 40
4 Joshi, B S and Kamat, V N (1972) *Indian J Chem* **10**, 771
5 Krishnaswamy, N R and Prasanna, S (1975) *Phytochemistry* **14**, 1663
6 Garg, V K and Nes, W R (1984) *Phytochemistry* **23**, 2925
7 Manzoor-i-Khuda, M (1966) *Tetrahedron* **22**, 2377
8 Bolger, L M, Rees, H H, Ghisalberti, E L, Goad, L J and Goodwin, T W (1970) *Tetrahedron Letters* 3043
9 Bolger, L M, Rees, H H, Ghisalberti, E L, Goad, L J and Goodwin, T W (1970) *Biochem J* **118**, 197
10 Subramanian, S S, Nair, A G R and Vedantham, T N C (1973) *Phytochemistry* **12**, 2078
11 Joshi, K C, Singh, P and Mehra, A (1979) *Planta Med* **37**, 64
12 Joshi, K C, Prakash, L and Shah, R K (1977) *J Indian Chem Soc* **54**, 1104
13 Prakash, L and Garg, G (1980) *Pharmazie* **35**, 500
14 Cattel, L, Balliano, G and Caputo, O (1980) *Planta Med* **37**, 264
15 Sucrow, W, Slopianka, M and Kircher, H W (1976) *Phytochemistry* **15**, 1533
16 Sucrow, W and Reimerdes, A (1968) *Z Naturforsch* **23b**, 42
17 Iida, T, Jeong, T M, Tamura, T and Matsumoto, T (1980) *Lipids* **15**, 66
18 Itoh, T, Kikuchi, Y, Tamura, T and Matsumoto, T (1981) *Phytochemistry* **20**, 761
19 Itoh, T, Yoshida, K, Tamura, T and Matsumoto, T (1982) *Phytochemistry* **21**, 727
20 Garg, V K and Nes, W R (1984) *Phytochemistry* **23**, 2919
21 Sinha, N K, Pandey, V B, Shah, A H and Dasgupta, B (1980) *Indian J Pharm Sci* **42**, 96
22 Abdul-Alim, M A (1971) *Planta Med* **19**, 318
23 Abdul-Alim, M A, Aboulezz, A F, Fayez, M B E and Seedhom, A E (1966) *Z Analyt Chem* **217**, 268
24 McKean, M L and Nes, W R (1977) *Phytochemistry* **16**, 683
25 Nes, W R (1977) *Advances in Lipid Research* (Kritchevsky, D and Paoletti, R, eds) Vol 15, p 233 Academic Press, New York
26 Balliano, G, Caputo, O, Viola, F, Delprino, L and Cattel, L (1983) *Lipids* **18**, 302

Phytochemistry, Vol 24, No 5, pp 1097–1099, 1985
Printed in Great Britain

0031–9422/85 $3 00 + 0 00

ON THE REPORTED OCCURRENCE OF A FUSICOCCIN CONJUGATE IN MAIZE COBS

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(*Received* 30 *July* 1984)

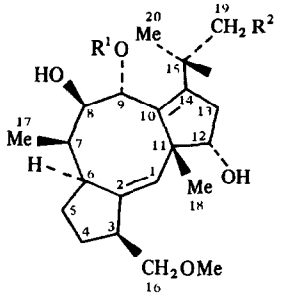
Key Word Index—*Zea mays*, Gramineae, cobs, fusicoccin, phytotoxin, plant growth substance

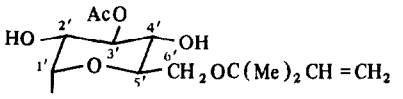
Abstract—The occurrence in immature maize cobs of a fusicoccin derivative, reported in 1980 by Russian workers, could not be confirmed Extraction and fractionation procedures were identical to those used by the Soviet authors and the analysis of the fractions mainly relied on a very sensitive and specific radioimmunoassay Possible reasons for these contradictory results are discussed

INTRODUCTION

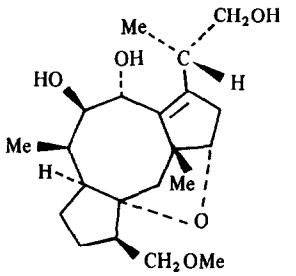
The fungal metabolite fusicoccin (**1**) has many biological activities typical of plant hormones [1], but unlike them its effects are tissue non-specific and its metabolic stability in plant tissues is remarkably high [2] Furthermore, in contrast with what is expected for a phytohormone, its distribution in nature appears to be very restricted [3] Nonetheless, the number of plants responding *in vivo* to, and binding *in vitro* with fusicoccin is quite high [3], suggesting that one or more metabolites capable of interacting with fusicoccin-binding sites are present in higher plants [4] This has found experimental support from investigations which are still in progress in our group [3]

In 1980 Muromtzev *et al* [5] reported the occurrence in immature maize cobs of a fusicoccin-like compound and proposed that fusicoccins are a new type of phytohormone Thus, for the first time a fusicoccin was detected in



1 R^1=  R^2 = OAc

2 R^1 = H R^2 = OH



3

higher plants, other than almond or peach infected by the pathogenic fungus *Fusicoccum amygdali* Del [6], a finding that raises the question of a physiological role of fusicoccin not considered before In particular, the observation that the fusicoccin-like compound is devoid of biological activity but becomes active on acid hydrolysis is suggestive of a modulation mechanism for fusicoccin activity

We decided to repeat the work of the Soviet authors with the purposes of elucidating the structure of the fusicoccin-like substance and of investigating its biological properties *in vitro* and *in vivo* The decision was also influenced by the inconsistency of some chemical results reported in the Soviet article with our long experience with the chemical reactivity of fusicoccin and its derivatives A preliminary report of this work has been published [7]

RESULTS

The acetone extract of immature maize cobs was prepared and processed according to the flow sheet of Muromtzev *et al* [5] to afford seven fractions after silica gel chromatography The fractions were hydrolysed and analysed by TLC under conditions identical to those used by the Soviet authors While some of the hydrolysed fractions contained sitosterol, as reported in ref [5], none showed a spot attributable to fusicoccin or to products arising from fusicoccin on acid hydrolysis When the hydrolysis was repeated on the fractions supplemented with authentic fusicoccin the analysis by TLC showed the total disappearance of this compound and the appearance of spots corresponding to the fusicoccin aglycone (**2**) and to its tetracyclic isomer (**3**) [8] Fusicoccin added to the fractions, both before and after acid hydrolysis, could be easily detected by TLC and had the same R_f as the authentic compound

In order to increase the analytical sensitivity a radioimmunoassay (RIA), which can quantitatively detect picomoles of fusicoccin and its derivatives [9], was used Again, negative results were consistently obtained when the crude extract of maize cobs and the fractions prepared from it by column chromatography were assayed by this method, both before and after hydrolysis Authentic fusicoccin added to the fractions was fully recovered by RIA, after hydrolysis the fusicoccin supplemented fractions still gave a positive result, in agreement with the presence of **2** and **3**, both of which are recognized by the antibodies [10]

DISCUSSION

The paper of Muromtzev *et al* [5] contains some data which can not be reconciled with our results The Soviet authors claim that an uncharacterized substance present in one of the fractions obtained by column chromatography of maize cobs extract affords on acid hydrolysis sitosterol and a bioactive product which behaves as fusicoccin on TLC run with three different solvent systems, and shows a mass spectrometric fragmentation

pattern with peaks characteristic of the monoacetylated aglycone moiety of fusicoccin The latter result is confirmed by the mass spectra of the deuteroacetyl and trimethylsilyl derivatives of the acid hydrolysis product

On the grounds of our long experience with fusicoccin and its derivatives [11], the finding of an *O*-acetyl group in the carbotricyclic moiety of a fusicoccin-like product obtained by hydrolysis with 0 6 N HCl at reflux for 40 hr is unexpected We repeated the hydrolysis on fusicoccin and on a minor metabolite of *F amygdali* which has an *O*-acetyl on C-12 instead of C-19 [12], and in both cases we observed on TLC the complete disappearance of the starting compound and the formation of the aglycone (2) and its tetracyclic isomer (3) Consequently, it is difficult to see how a true fusicoccin derivative could survive the conditions used by Muromtzev *et al* for the hydrolysis [5] At this point the structural elucidation of the new maize metabolite becomes highly desirable

Unfortunately, our attempts to isolate this metabolite failed, since we could not detect any substance related to fusicoccin in a maize cob extract The extraction and fractionation procedures of the plant tissue were identical to those used by the Soviet authors and the methods adopted for the detection of a fusicoccin derivative were highly sensitive The latter methods relied on TLC with different solvent systems and on a specific RIA [9] which can detect fusicoccin and most of its acid hydrolysis derivatives at the picomolar level It was already known that the fusicoccin aglycone (2) and its tetracyclic isomer (3) are recognized by the antifusicoccin antibodies [10], and in the present investigation it has been ascertained that two other derivatives arising from fusicoccin in acid media, namely de-*t*-pentenylfusicoccin and de-*t*-pentenyl-dideacetylfusicoccin, interact with the antibodies as efficiently as fusicoccin Thus it appears that a fusicoccin derivative of the type reported by Muromtzev *et al* [5] could hardly have escaped detection in our investigation As reported above our endeavour to detect fusicoccin or a fusicoccin derivative in the crude extract or in the chromatographic fractions, both before and after acid hydrolysis, failed In order to reconcile this negative result with those of the Soviet authors it is necessary to postulate that the fusicoccin-like compound is not present in all varieties of maize, a situation which anyhow strongly limits the physiological role of the compound In this context it is noteworthy that compounds responding to fusicoccin antibodies are absent in extracts of almond leaves, peach fruits and maize roots (unpublished results)

EXPERIMENTAL

Silica gel 60 (0 05–0 2 mm) was used for CC TLC was performed with silica gel F_{254} precoated plates (0 25-mm layer) and the following solvent systems $CHCl_3$–*iso*-PrOH (9 1), C_6H_6–*iso*-PrOH (9 1), EtOAc The plates were sprayed with 10% H_2SO_4 in MeOH and then heated at 110° for 5 min Radioimmunoassays (RIA) were carried out according to ref [9]

Extraction and fractionation Zea mays L, variety XL 342 (Italian Dekalb, Mestre, Italy), was grown in the field and cobs were collected in the milky stage of ripening in July after 5 months from seed The cobs (1 kg) were first cut into small pieces and then homogenized with 500 ml H_2O in a blender The thick suspension was macerated at room temp for 2 hr with Me_2CO (1 1), filtered, the solids extracted again at room temp with Me_2CO (1 1) and filtered The organic solvent was removed from the pooled filtrates under red pres and the aq layer extracted with $CHCl_3$ (3 × 200 ml) Evaporation under red pres of the dried (Na_2SO_4) $CHCl_3$ extract gave a greenish residue (2 7 g) which was dissolved in C_6H_6–petrol (1 1) and adsorbed on a silica gel column (2 5 × 80 cm) packed in the same solvent mixture Stepwise elution was performed as in [5] with the following solvents C_6H_6–petrol (1 1), C_6H_6, C_6H_6–$CHCl_3$ (1 1), $CHCl_3$, $CHCl_3$–*iso*-PrOH (9 1), EtOAc, EtOH One litre of each solvent was passed through the column, except for $CHCl_3$ (1 7 l) The fractions were evapd under red pres to afford oily residues The wt recovered was 2 44 g (90 5% yield)

Hydrolysis of the fractions Each fraction was refluxed 40 hr with 0 6 N HCl in MeOH After cooling the solution was neutralized with 4 N NaOH, the solvent evapd under red pres and the residue extracted 3 times with EtOAc The pooled extracts were dried (Na_2SO_4), evapd under red pres, and analysed by TLC with three solvent systems in parallel with appropriate reference compounds Neither fusicoccin nor any acid hydrolysis derivatives were detectable, β-sitosterol was present in the fractions eluted with $CHCl_3$, $CHCl_3$–*iso*-PrOH, EtOAc and EtOH The same procedure was used for the analysis of the fractions supplemented with fusicoccin

Acknowledgements—This work was supported in part by the Ministry of Education (Rome), and in part by the Italian National Research Council (CNR), special *ad hoc* programme 'Chimica fine e secondaria', subproject Ca.

REFERENCES

1 Marrè, E (1979) *Ann Rev Plant Physiol* **30**, 273
2 Ballio, A, Federico, R and Scalorbi, D (1977) *Rend Accad Naz Lincei* **63**, 604
3 Aducci, P, Ballio, A, Federico, R and Montesano, L (1982) in *Plant Growth Substances* (Wareing, P F, ed) p 395 Academic Press, London
4 Aducci, P, Crosetti, G, Federico, R and Ballio, A (1980) *Planta* **148**, 208
5 Muromtzev, G S, Kobrina, N S, Voblikova, V D and Koreneva, Y M (1980) *Izv Akad Nauk SSSR, Ser Biol* **2**, 897
6 Ballio, A, D'Alessio, V, Randazzo, G, Bottalico, A, Graniti, A, Sparapano, L, Bosnar, B, Casinovi, C G and Gribanovski-Sassu, O (1976) *Physiol Plant Pathol* **8**, 163
7 Aducci, P, Ballio, A, Federico, R, Evidente, A, Iasiello, I and Randazzo, G (1983) *Phytopath Med* **22**, 95
8 Casinovi, C G, Santurbano, B, Conti, G, Malorni, A and Randazzo, G (1974) *Gazz Chim Ital* **104**, 679
9 Federico, R, Aducci, P, Marra, M and Pini, C (1981) *Z Pflanzenphysiol* **104**, 275
10 Pini, C, Vicari, G, Ballio, A, Federico, R, Evidente, A and Randazzo, G (1979) *Plant Sci Letters* **16**, 343
11 Ballio, A (1981) in *Toxins in Plant Disease* (Durbin, R D, ed) p 395 Academic Press, New York
12 Ballio, A, Casinovi, C G, Framondino, M, Grandolini, G, Randazzo, G and Rossi, C (1972) *Experientia* **28**, 1150