Pergamon 0031-9422(95)00505-6 Phytochemistry, Vol. 40, No. 6, pp. 1637–1641, 1995

0031-9422/95 $9.50 + 0.00

PUTAMINOXIN, A PHYTOTOXIC NONENOLIDE FROM *PHOMA PUTAMINUM*

ANTONIO EVIDENTE,*† ROSA LANZETTA,‡ RENATO CAPASSO,* ANNA ANDOLFI,* ANTONIO BOTTALICO,§ MAURIZIO VURRO¶ and MARIA CHIARA ZONNO¶

*Dipartimento di Scienze Chimico-Agrarie, Università di Napoli "Federico II", Via Università 100, 80055 Portici, Italy; ‡Dipartimento di Chimica Organica e Biologica, Università di Napoli "Federico II", Via Mezzocannone 16, 80134 Napoli, Italy; §Istituto di Patologia Vegetale, Università degli Studi di Sassari, Via E. De Nicola, 07100 Sassari, Italy; ¶Istituto Tossine e Micotossine da Parassiti Vegetali, CNR, Viale L. Einaudi 51, 70125 Bari, Italy

(*Received in revised from* 24 *May* 1995)

Key Word Index—*Erigeron annuus*; Compositae; Phoma putaminum; fungus; phytotoxins; macrolides; nonenolides; putaminoxin.

Abstract—*Phoma putaminum*, the causal agent of leaf necrosis of *Erigeron annuus*, a common weed of field and pasture, produced toxic metabolites when grown in liquid culture. The main phytotoxin, named putaminoxin, was isolated and characterized using spectroscopic and chemical methods as (5*S*)5-hydroxy-9-propyl-6-nonen-9-olide, a new 10-macrolide. When assayed on leaves of host and non-host plants, putaminoxin showed a wide range of toxicity, with leaves of *E. annuus* being most sensitive.

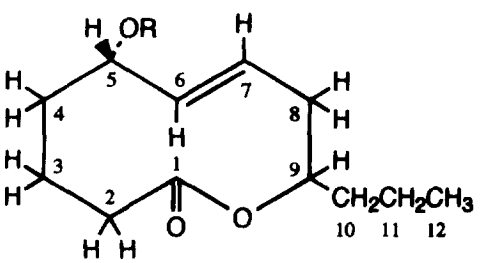
INTRODUCTION

Erigeron annuus, commonly named annual fleabane, is an indigenous weed from North America widely found in field and pastures all over Europe, including Italy. Studies on the possible use of weed fungal pathogens for biological control of noxious plants, led us to collect diseased leaves of *E. annuus* showing necrotic spots, surrounded by chlorotic haloes. A fungus, identified as *Phoma putaminum*, was isolated from the diseased leaves. Considering that phytotoxins may also be directly used as herbicides or as analogues for the development of selective non-persistent herbicides [1], research was carried out to isolate and characterize the toxic metabolites produced *in vitro* by *P. putaminum*. Culture filtrates of *P. putaminum* proved to be phytotoxic, causing wide range of leaf necrosis on both host and non-host plants.

The present paper describes the isolation and chemical and biological characterization of the main phytotoxin produced by *P. putaminum*, named putaminoxin, which is a new disubstituted nonenolide.

RESULTS AND DISCUSSION

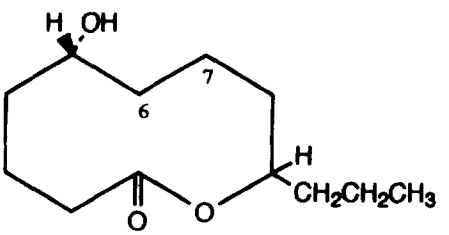
Phytotoxic culture filtrates of *P. putaminum* were extracted with EtOAc giving an oily residue which was purified by a combination of CC and TLC, as described in detail in the Experimental, in order to yield putaminoxin (**1**), as a homogeneous oily compound without-



R

1 H

2 Ac



3

standing recrystallization. When it was assayed on host leaves using a puncture assay, putaminoxin (20 μg per droplet) caused chlorosis, followed 2 days later by necrosis. Assayed on leaves of weed species and on non-host

†Author to whom correspondence should be addressed.

Table 1. Effect of putaminoxin on a range of various plant species using a leaf-puncture assay

Common name	Latin name	Family	Toxicity*
Annual fleabane	*Erigeron annuus*	Compositae	+ + +
Annual dog's mercury	*Mercurialis annua*	Euphorbiaceae	+ + +
Annual sowthistle	*Sonchus oleraceus*	Compositae	+
Clover	*Trifolium pratense*	Leguminosae	−
Chickweed	*Stellaria media*	Caryophyllaceae	−
Fat-hen	*Chenopodium album*	Chenopodiaceae	+
Globe artichoke	*Cynara cardunculus*	Compositae	+ +
Mandarin	*Citrus aurantium*	Rutaceae	−
Nettle	*Urtica* spp.	Urticaceae	+ +
Parsley	*Petroselinum crispum*	Umbelliferae	Chlorosis
Strawberry	*Fragaria vesca*	Rosaceae	+
Sweet basil	*Ocimum basilicum*	Labiatae	−
Swiss chard	*Beta vulgaris*	Chenopodiaceae	−
Tomato	*Solanum lycopersicum*	Solanaceae	Chlorosis

*Toxicity index determined on the following scale: −, no symptoms; +, necrosis (0–1 mm); + +, necrosis (1–3 mm); + + +, necrosis (3–5 mm). Droplets (10 μl) of toxin solution (2 $\mu g\,\mu l^{-1}$) were applied on previously needle-punctured leaves. Effects were observed 2 days after droplet application.

cultivated plants, putaminoxin also showed a range of toxicities, mandarin and sweet basil being among the less sensitive, and annual dog's mercury the most sensitive (Table 1). However, the toxicity observed on annual fleabane was the most severe. The toxin also showed a weak toxicity (at 100 μg) toward *Geotrichum candidum*, whereas when assayed up to 100 μg against both *Escherichia coli* and *Bacillus subtilis* it was not toxic. Moreover, it showed no activity against *Artemia salina* larvae when assayed up to 2×10^{-4} M.

Putaminoxin had a molecular formula of $C_{12}H_{20}O_3$ as deduced from its HR EI mass spectrum, giving a total of three degrees of unsaturations. Its IR spectrum showed bands for hydroxyl, olefinic and ester carbonyl groups [2, 3], suggesting that this toxin has a lactone nature. The UV spectrum showed the absence of chromophores; therefore, the carbonyl lactone and the double bond group should be unconjugated. These structural features were confirmed by a careful examination of the ^{1}H and ^{13}C NMR spectra (Table 2). A doublet of double doublets (H-7) and a double doublet (H-6) typical of two protons of a *trans*-disubstituted ($J_{6,7} = 15.4$ Hz) olefinic group [3–5] were observed at δ5.52 and 5.30, respectively. As shown by the COSY spectrum [6], H-6 coupled with the proton of a secondary hydroxylated carbon resonating as a doublet of double doublets at δ3.98 (H-5). When the spectrum of 1 was recorded in DMSO-d_6, the signal of H-5 appeared as a very complex multiplet at δ3.75, because it was further coupled with the proton of the geminal alcohol group (HOC-5) present as a doublet ($J = 3.6$ Hz) at δ4.65. As expected, the latter disappeared on exchange with D_2O and H-5 changed into a doublet of double doublets [5]. This proton (H-5) was also coupled with the protons of the adjacent methylene group (H_2C-4) resonating both as multiplet at δ1.99 and 1.52, respectively. The other olefinic proton, H-7, also coupled with the protons of the adjacent methylene group (H_2C-8) appearing as a doublet of double doublets and a multiplet at δ2.33 and 1.89, respectively. The same H_2C-8, in turn, correlated with the proton (H-9) of another secondary oxygenated carbon appearing as a very complex multiplet at δ5.0. C-9 represents the closure point of the macrocyclic ring and the complexity of the geminal proton (H-9) was justified by its further coupling with the adjacent methylene group (H_2C-10) of the attaching propyl side-chain whose complete proton

Table 2. ^{1}H and ^{13}C NMR ($CDCl_3$) of putaminoxin (1). Chemical shift are in δ using solvent as internal standard

Position number	δ_C	m†	δ_H	m	J(Hz)
1	175.8	*s*			
2	35.6	*t*	2.42	*ddd*	14.6, 7.8, 4.0
2′			1.99	*m*	
3	22.2	*t*	1.90	*m*	
3′			1.90	*m*	
4	38.7	*t*	1.99	*m*	
4′			1.52	*m*	
5	74.0	*d*	3.98	*ddd*	10.0, 9.4, 3.3
6	137.2	*d*	5.30	*dd*	15.4, 9.4
7	131.5	*d*	5.52	*ddd*	15.4, 10.6, 4.8
8	40.3	*t*	2.33	*ddd*	12.4, 4.8, 4.0
8′			1.89	*m*	
9	75.3	*d*	5.00	*m*	
10	36.3	*t*	1.69	*m*	
10′			1.52	*m*	
11	19.1	*t*	1.40	*m*	
11′			1.40	*m*	
12	13.8	*q*	0.91	*t*	7.2

*2D ^{1}H, ^{1}H and ^{13}C, ^{1}H experiments delineated the correlation of all protons and the corresponding carbons.

†Multiplicities determined by DEPT.

chemical shift assignments are shown in Table 2. Finally, the protons which are typical of a methylene located α with respect to a carbonyl group (H_2C-2) were present as a doublet of double doublets and a multiplet at δ2.42 and 1.99, respectively [3, 5].

Considering these results and the molecular formula of putaminoxin ($C_{12}H_{20}O_3$), a furthermore methylene group was yet to be located. The ^{13}C NMR data (Table 2) and the correlations found in the 2D ^{13}C, ^1H NMR experiment [6] confirmed both the partial structures discussed above, especially the lactone nature of the macrocyclic ring (O=C-1 at δ175.8), and corroborated the presence of this further methylene group (H_2C-3), which resonated at δ22.2 and was located β with respect to the lactone carbonyl group [3, 6]. From these data, putaminoxin proved to be a new disubstituted nonenolide and may be formulated as 5-hydroxy-9-propyl-6-nonen-9-olide (**1**).

The phytotoxin structure was supported by the peaks observed in the mass spectrum of **1** generated by fragmentation mechanisms typical of macrolides [7] and by the presence of a propyl side-chain at C-9 [5]. The $[M]^+$ (*m/z* 212.1405, $C_{12}H_{20}O_3$), which consecutively lost C_2H_4 and CO_2, produced ions at *m/z* 184 and 140.1207 ($C_9H_{16}O$, base peak). The latter, corresponding to an intermediate ion, probably a 3-hydroxy-5-propylcyclohexene ion, generated the ions at *m/z* 125 and107 or 97, respectively, by alternative loss of Me followed by H_2O or $CH_2CH_2CH_3$. Moreover, by an alternative fragmentation pathway, the $[M]^+$, which consecutively lost OH and CH_2CH_3, yielded the ions at *m/z* 195 and 166, respectively.

The structure of putaminoxin was confirmed by preparing two key derivatives, whose spectroscopic data were all consistent (see Experimental). By reaction with pyridine and acetic anhydride, **1** was converted to the corresponding 5-*O*-acetylderivative (**2**) ($[M]^+$ *m/z* 254 by EI mass spectrometry). The IR spectrum showed the absence of hydroxyl groups and the presence of a band typical of an acetyl carbonyl group [2, 3] at 1738 cm^{-1}. Its ^1H NMR differed from that of **1** essentially in the downfield shift (Δδ 1.10) of the doublet of double doublets of H-5 observed at δ5.08 and in the presence of the singlet of the acetyl group at δ2.0.

Catalytic hydrogenation of **1** confirmed the presence of the *trans*-double bond [C(6)=C(7)] yielding the corresponding 6,7-dihydroderivative (**3**) ($[M + H]^+$ *m/z* 215 by EI mass spectrometry, in agreement with the well-known behaviour of lactones [3]). Its IR spectrum showed the absence of bands due to the olefinic group, as well as the lack of typical signals in the ^1H and ^{13}C NMR spectra. Moreover, the ^1H NMR of **3**, compared to that of **1**, showed an upfield shift (Δδ0.43), as well as the increased complexity of the signal at H-5, which resonated as a multiplet at δ3.55, and the presence of more complex signals in the aliphatic region between δ2.0 and 1.3. The ^{13}C NMR differed from that of **1** in a significant upfield shift (Δδ4.4 and 10.3, respectively) of C-5 and C-8 at δ69.6 and 30.0, and in the presence of the signals of two more methylene groups at δ34.1 and 20.5 attributed to C-6 and C-7, respectively. Finally, the absolute configuration of the chiral carbinol C-5 centre was determined by the application of the Horeau's GC method [8]. The results obtained with the assumption that the double bond [C(6)=C(7)] is a larger substituent than the H_2C-4 [9] indicated an *S*-configuration at the optically active C-5 carbon.

In conclusion, the new disubstituted nonenolide structure of putaminoxin appear to have been demonstrated statisfactorily. Nonenolides are macrolides, a well-known group of naturally occurring compounds [10–13]. The biological activity of some of these compounds is such that their total synthesis has been achieved [14]. Macrolides are also fungal metabolites [13], such as pinolidoxin, a tetrasubstituted 5-nonen-9-olide [15], and the three related pinolidoxins [16] recently isolated by our group from *Ascochyta pinodes*, as well as the diplodialides A–D produced by *Diplodia pinea* [13], which are all structurally related to putaminoxin. Moreover, considering that the new phytotoxins could be used as herbicides or as analogues for the development of selective and safe herbicides, and considering the promising semi-selective toxic effects observed between plant species, further research is in progress in order to assess the selectivity of putaminoxin, as well as other metabolites produced by *P. putaminum*, which would appear to be structurally related to the main metabolite.

EXPERIMENTAL

General. Optical rotations: $CHCl_3$. IR and UV: neat and MeCN, respectively. ^1H and ^{13}C NMR: $CDCl_3$ at 400 and/or 270 MHz and 100 and/or 68 MHz, respectively, using solvent as int. standard. Carbon multiplicities were determined by DEPT spectra [6]. DEPT, COSY and 2D heteronuclear chemical shift correlation expts were performed using Bruker standard microprograms. EI and HR EIMS: 70 eV. Analytical and prep. TLC: silica gel (Merck, Kieselgel 60 F_{254}, 0.25 and 0.50 mm, respectively) or reverse-phase (Whatman, KC-18 F_{254}, 0.20 mm) plates; the spots were visualized by exposure to UV radiation and/or by spraying first with 10% H_2SO_4 in MeOH and then with 5% phosphomolybdic acid in MeOH, followed by heating at 110° for 10 min. CC: silica gel (Merck, Kieselgel 60, 0.063–0.20 mm); solvent systems: (A) $CHCl_3$–*iso*PrOH (19:1); (B) EtOAc–*n*-hexane (1.5:1); (C) $CHCl_3$–*iso*PrOH (9:1); (D) EtOH–H_2O (1.5:1); (E) $CHCl_3$–*iso*PrOH (32.3:1). (±)-α-Phenylbutyric anhydride was purchased from Fluka. GC analyses were carried out on a Supelco capillary column (30 m × 0.25 mm) with He at 1 ml min^{-1} and isothermal 200° using a dual FID detector.

Production, extraction and purification of putaminoxin (**1**). Cultures of freshly isolated *P. putaminum* Speg. were obtained from diseased leaves of *E. annuus* (L.) Pers. and single spore cultures maintained on potato dextrose agar (PDA) medium, with frequent subculturing at monthly intervals. Toxic metabolites were produced by using 1 ml of an abundant conidial suspension to inoculate 200 ml

(in 11 Erlenmeyer flasks) of M-1-D medium [17]. The shaken (200 rpm) cultures were incubated at 25° for 8 days, then filtered and lyophilized. The lyophilized material obtained from culture filtrates (12 l) was resuspended in dist. H_2O (1 l), acidified to pH2 with HCO_2H and extracted with EtOAc (3 × 1 l). Organic solvent extracts were combined, dried (Na_2SO_4) and evapd under red. pres. The brown oily residue (3.57 g), which had high phytotoxic activity, was fractionated by CC eluted with solvent system A to yield 9 groups of homogeneous frs. Pooled fr. groups between 1–6 and 8 showed phytotoxic activity. The residue (91.1 mg) left from group 4, containing the main metabolite (R_f 0.41 and 0.44 by TLC on silica gel, eluent A and on reverse-phase, eluent D, respectively) was further purified by two successive prep. TLC steps (silica gel, eluents B and C, respectively) producing crude metabolite (30 mg). This was finally purified by prep. TLC on reverse-phase (eluent D) yielding putaminoxin (**1**) as a homogeneous oily compound (24 mg) withstanding recrystallization. A further amount of **1** (5.2 mg; total 2.4 mg l^{-1}) was obtained from fr. group 5 of the initial column using the same purification procedure.

Putaminoxin (**1**). $[\alpha]_D^{25}$ − 23.1 (*c* 1.6). UV λ_{max} nm (log ε) < 220. IR ν_{max} cm^{-1}: 3402 (OH), 1729 (C=O), 1667 (C=C), 1182 (O–CO). ^1H and ^{13}C NMR spectra: Table 2. EI MS, *m/z* (rel. int.): 212.1405 ($C_{12}H_{20}O_3$, calcd 212.1413) $[M]^+$ (19), 195 $[M - OH]^+$ (9). 184 $[M - C_2H_4]^+$ (7), 166 $[M - OH - C_2H_5]^+$ (15), 140.1207 ($C_9H_{16}O$, calcd 140.1201) $[M - C_2H_4 - CO_2]^+$ (100), 125 $[M - C_2H_4 - CO_2 - Me]^+$ (73), 107 $[M - C_2H_4 - CO_2 - Me - H_2O]^+$ (28), 97 $[M - C_2H_4 - CO_2 - C_3H_7]^+$ (17).

5-O-*Acetylputaminoxin* (**2**). Putaminoxin (**1**, 3.1 mg) was acetylated with pyridine (100 μl) and Ac_2O (100 μl) at room temp. overnight. The oily residue left by the reaction work-up was purified by prep. TLC (silica gel, eluent A) to give **2** as a homogeneous compound (3.4 mg). UV λ_{max} nm (log ε) < 220. IR ν_{max} cm^{-1}: 1738 (C=O), 1732 (C=O), 1667 (C=C), 1260 (O–CO), 1096, 1022 (O–CO). ^1H NMR differed from that of **1** in the following signals. δ5.08 (1H, *ddd*, *J* = 10.2 Hz, 9.4 Hz and 3.0 Hz, H-5), 2.00 (3H, *s*, MeCO). EIMS, *m/z* (rel. int.): 254 $[M]^+$ (14), 212 $[M - CH_2CO]^+$ (21), 195 $[M - AcO]^+$ (22), 194 $[M - AcOH]^+$ (51), 182 $[M - CH_2CO - C_2H_6]^+$ (85), 167 $[M - AcO - C_2H_4]^+$ (27), 164 $[M - AcOH - C_2H_6]^+$ (72), 140 $[M - CH_2CO - C_2H_4 - CO_2]^+$ (100), 125 $[M - CH_2CO - C_2H_4 - CO_2 - Me]^+$ (95), 107 $[M - CH_2CO - C_2H_4 - CO_2 - Me - H_2O]^+$ (59), 97 $[M - CH_2CO - C_2H_4 - CO_2 - C_3H_7]^+$ (50).

6,7-*Dihydroputaminoxin* (**3**). Putaminoxin (**1**, 5.3 mg) in MeOH (2.5 ml) was added to a presaturated PtO_2 (5 mg) suspension in the same solvent (2.5 ml) and hydrogenated at room temp. and atm. pres. with stirring. After 1 hr, the reaction was stopped by filtration, evapd under red. pres. and the residue purified by CC (eluent E) to give **3** as a homogeneous oily compound (3.8 mg). UV λ_{max} nm (log ε) < 220. IR ν_{max} cm^{-1}: 3419 (OH), 1724 (C=O), 1264 (O–CO). ^1H and ^{13}C NMR differed from those of **1** in the following signal systems: ^1H NMR, δ3.55 (1H, *m*, H-5), 2.52 (1H, *ddd*, *J* = 15.7 Hz, 4.4 Hz and 2.9 Hz, H-2), 2.23 (1H, *ddd*, *J* = 15.7 Hz, 11.7 Hz and 2.6 Hz, H-2'), 2.0–1.3 (14H, *m*, H_2C-3, H_2C-4, H_2C-6, H_2C-7, H_2C-8, H_2C-10 and H_2C-11); ^{13}C NMR, δ69.6 (*d*, C-5), 35.3 (*t*, C-4) 34.1 (*t*, C-6) 30.0 (*t*, C-8), 20.5 (*t*, C-7). EIMS, *m/z* (rel. int.): 215 $[M + H]^+$ (7), 214 $[M]^+$ (0.5), 196 $[M - H_2O]^+$ (8), 186 $[M - C_2H_4]^+$ (6), 168 $[M - H_2O - C_2H_4]^+$ (28), 143 $[M + H - C_2H_4 - CO_2]^+$ (42), 142 $[M - C_2H_4 - CO_2]^+$ (37), 127 $[M - C_2H_4 - CO_2 - Me]^+$ (98), 109 $[M - C_2H_4 - CO_2 - Me - H_2O]^+$ (98), 99 $[M - C_2H_4 - CO_2 - C_3H_7]^+$ (100).

Configuration of carbinol C-5 centre in **1**. A dried putaminoxin (**1**, 2 mg) sample in dry pyridine (7 μl) was treated with dist. racemic (±)-α-phenylbutyric anhydride (6.2 μl) for 1 hr at 40°. The reaction mixt. was worked-up [8] and the product analysed by GC using the conditions described above.

Biological methods. Each sample was dissolved in a small amount of MeOH and brought to the required concn with dist. H_2O or sea H_2O soln (brine shrimp assay).

Leaf-puncture assay on host plants. Phytotoxic activity of liquid culture filtrates chromatographic frs or pure toxin was tested using an *in vivo* assay on host leaves. *Erigeron annuus* plants were grown in a growth chamber at 22°, using a light–dark cycle of 14–10 hr, with a high level of moisture. Undetached and fully expanded young leaves of 1-month-old plants were used, applying 10 μl of test solns to previously needle-punctured sites on the leaves. Plants were then covered with a glass dome to avoid droplet drying. Effects were observed 2 days after droplet application. Whole culture filtrate was tested using droplets of 10 μl, whereas frs were first dissolved in a small amount of MeOH (0.4 μl), and then brought up to the final concn. Pure toxin was tested up to 20 μg per droplet.

Leaf-puncture assay on non-host plants. Phytotoxic activity of putaminoxin was also tested by a leaf-puncture assay, using young detached leaves of another 13 species, according to the method described in ref. [18]. Putaminoxin was tested at 20 μg per droplet.

Antifungal activity. Antifungal activity was assayed on *Geotrichum candidum* according to the method previously described [19]. Putaminoxin was tested up to 100 μg per disc.

Antibiotic activity. Antibacterial activity was tested on *Bacillus subtilis* and *Escherichia coli* according to methods described previously [20]. Putaminoxin was assayed up to 100 μg per disc.

Mycotoxic activity. Zootoxic activity was tested on brine shrimp larvae according to the method described in ref. [19]. Pure toxin was tested up to 2×10^{-4} M.

Acknowledgements—This research was aided by grants from the Italian Ministry for University and Scientific and Technological Research and, in part, by the National Research Council of Italy (CNR). The MS data were provided by 'Servizio di spettrometria di massa del CNR e dell'Università di Napoli Federico II'; assistance of the staff is gratefully acknowledged. The authors thank the

'Centro Interdipartimentale di Metodologie Chimico-Fisiche ed il Centro Interdipartimentale di Analisi Strumentale dell'Università di Napoli Federico II' for NMR and HR EIMS spectra, respectively. Contribution N. 112 (DISCA).

REFERENCES

1. Strobel, G. A., Sugawara, F. and Clardy, J. (1987) in *Allelochemicals: Role in Agriculture and Forestry* (Waller, G. R., ed.), pp. 516–523. ACS Symposium Series 330, Washington, DC.
2. Nakanishi, K. and Solomon, P. H. (1977) in *Infrared Absorption Spectroscopy* (2nd edn), pp. 17–21, 25–30, 38–44. Holden-Day, Oakland.
3. Pretsch, E., Seibl, J., Simon, D. and Clerc, T. (1989) in *Table of Spectral Data for Structure Determination of Organic Compounds* (Fresenius, W., Huber, J. K. F., Punger, E., Rechnitz, G. A., Simon, W. and West, Th. S., eds), pp. C195, H130, H205, H210, H215, H220, I30, I35, I36, I135, I140, M240. Springer-Verlag, Berlin.
4. Sternhell, S. (1969) *Quart. Rev.* **23**, 237.
5. Silverstein, R. M., Bassler, C. G. and Morrill, T. C. (1974) in *Spectrometric Identification of Organic Compounds*, pp. 19–23, 174–175, 211–218. J. Wiley & Sons Inc., New York.
6. Breitmaier, E. and Voelter, W. (1987) in *Carbon-13 NMR Spectroscopy*, pp. 43–47, 73–106, 194–196, 206–213, 215–232. VCH-Verlagsgesellshaft, Weinheim.
7. Porter, Q. N. (1985) in *Mass Spectrometry of Heterocyclic Compounds* (2nd edn), pp. 260–278. J. Wiley & Sons Inc., New York.
8. Brooks, J. W. C. and Gilbert, J. D. (1973) *J. Chem. Soc., Chem. Commun.* 194.
9. Fiaud, J. C., Horeau, A. and Kagan, H. B. (1977) in *Stereochemistry Fundamentals and Methods* (Vol. 3) (Kagan, H. B., ed), pp. 64–65. Georg Thieme Publishers, Suttgart.
10. Dean, F. M. (1963) in *Naturally Occurring Oxygen Ring Compounds*, pp. 553–554. Butterworth, London.
11. Richards, J. M. and Hendrickson, J. B. (1964) in *The Biosynthesis of Steroids, Terpenes and Acetogenins*, pp. 28–31. W. A. Benjamin, New York.
12. Manitto, P. (1981) in *Biosynthesis of Natural Products*, pp. 208–210. Ellis Harwood, Chicester.
13. Turner, W. B. and Aldridge, D. C. (1983) in *Fungal Metabolites II*, pp. 104–108, 505. Academic Press, London.
14. Thomson, R. H. (1985) in *The Chemistry of Natural Products*, pp. 91–106. Blackie, Glasgow (and refs cited therein).
15. Evidente, A., Lanzetta, R., Capasso, R., Vurro, M. and Bottalico, A. (1993) *Phytochemistry* **34**, 999.
16. Evidente, A., Capasso, R., Abouzeid, M. A., Lanzetta, R., Vurro, M. and Bottalico, A. (1993) *J. Nat. Prod.* **56**, 1937.
17. Pinkerton, F. and Strobel, G. A. (1987) *Proc. Natl Acad. Sci. USA* **73**, 4007.
18. Sugawara, F., Strobel, G. A., Fisher, L. E., Van Duyne, G. D. and Clardy, J. (1985) *Proc. Natl Acad. Sci. USA* **82**, 8291.
19. Bottalico, A., Logrieco, A. and Visconti, A. (1989) in *Fusarium: Mycotoxins, Taxonomy and Pathogenicity* (Chelkowsky, J., ed.), pp. 85–119. Elsevier, Amsterdam.
20. Bottalico, A., Capasso, R., Evidente, A., Randazzo, G. and Vurro, M. (1990) *Phytochemistry* **29**, 93.