Phytochemistry, Vol. 28, No. 5, pp. 1303–1305, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00

JASMONIC ACID-LIKE SUBSTANCES FROM THE CULTURE FILTRATE OF *BOTRYODIPLODIA THEOBROMAE*

OTTO MIERSCH, JÜRGEN SCHMIDT, GÜNTHER SEMBDNER and KLAUS SCHREIBER

Institute of Plant Biochemistry, Academy of Sciences of the G.D.R., 4050 Halle (Saale), Weinberg 3, G.D.R.

(*Received* 20 *September* 1988)

Key Word Index—*Botryodiplodia theobromae*; Sphaeropsidaceae; (+)-4,5-didehydro-7-*iso*-jasmonic acid; ethyl (+)-7-*iso*-jasmonate; (1*R*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-propionic acid; (1*S*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-butyric acid.

Abstract—Four cyclopentanoidal fatty acids were isolated from the fungus *Botryodiplodia theobromae* and identified as jasmonic acid-like substances. Their structures are (+)-4,5-didehydro-7-*iso*-jasmonic acid [(1*R*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-4-en-1-yl-acetic acid], ethyl (+)-7-*iso*-jasmonate [ethyl (1*R*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-acetate], (1*R*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-propionic acid and (1*S*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-butyric acid.

INTRODUCTION

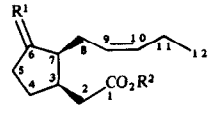
Botryodiplodia theobromae Pat. is a common tropical fungus known to produce several cyclopentanoidal fatty acids with plant growth regulating activities [1, 2]. Major substances are (+)-7-iso-jasmonic acid (**1**) and (−)-jasmonic acid (**5**). Some other metabolites possessing the 7-*iso*-configuration have already been described as (+)-11,12-didehydro-7-*iso*-jasmonic acid, (+)-9,10-dihydro-7-*iso*-jasmonic acid and cucurbic acid [2].

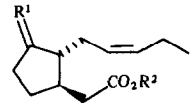
In continuation of the phytochemical investigation of this fungus we now report the isolation of four further compounds, one of which is new and the three others described for the first time as metabolites of *Botryodiplodia theobromae*.

RESULTS AND DISCUSSION

From a surface culture of *Botryodiplodia theobromae* three acidic substances and one neutral compound were isolated and after chromatographic purification by CC, TLC and HPLC, identified by GC-MS, IR, optical rotation and ^{1}H NMR, respectively, combined with chemical modifications. The acidic extract contained in low yields (+)-4,5-didehydro-7-*iso*-jasmonic acid (**9**), (1*R*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)cyclopent-1-yl-propionic acid (**11**) and (1*S*,2*S*)-(+)-3-oxo-2-(2*Z*-pentenyl)-cyclopent-1-yl-butyric acid (**12**); the neutral compound was proved to be ethyl (+)-7-*iso*-jasmonate (**2**).

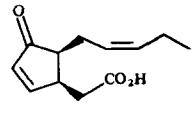
The identity of **2** was confirmed by GC-MS and comparison with authentic (−)-**6**. The key fragment at *m/z* 173 ($[M-OC_2H_5]^+$) represents the unique difference to the mass spectrum of **11**-methyl ester. Besides **2**, compound **6** was present as indicated by GC, but its natural occurrence has to be doubted because possible isomerization of **2** to **6** could not be excluded. The unsaturated acid **9** is accompanied by **10** in the ratio 4:1 as shown by TLC. Hydrogenation of **9**–**10** with sodium borohydride, reducing both the keto groups and the ring double bonds, gave the four isomers **3**, **4**, **7** and **8** [3].

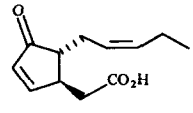




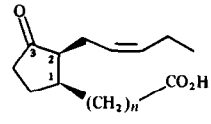
1 R^1 = O, R^2 = H
2 R^1 = O, R^2 = C_2H_5
3 R^1 = α-OH,β-H, R^2 = H
4 R^1 = α-H,β-OH, R^2 = H

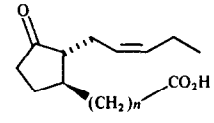
5 R^1 = O, R^2 = H
6 R^1 = O, R^2 = C_2H_5
7 R^1 = α-OH,β-H, R^2 = H
8 R^1 = α-H,β-OH, R^2 = H





9 **10**





11 n = 2
12 n = 3

13 n = 2
14 n = 3

Catalytic hydrogenation of the methyl esters gave 9,10-dihydro-**1** methyl ester and 9,10-dihydro-**5** methyl ester [2, 4, 5]. Like **6**, compound **10** might be an artifact originating from **9** by isomerization during the isolation procedure.

In the mass spectrum of **11**-methyl ester the fragments $[M-(CH_2)_2COOMe]^+$ and $[M-C_5H_8]^+$ indicate a *n*-propionic acid side chain at C-1 and a pentenyl side chain at C-2. The base peak at *m/z* 83 ($[C_5H_7O]^+$) stems from the cyclopentanone ring [6]. The *cis*-configuration of

both side chains was deduced from the positive optical rotation [7] and verified by the facile alkaline isomerization to **13**. The ^{1}H NMR spectrum, proving the *cis*-double bond, was very similar to that of **5** [6]. Hydrogenation with Adams catalyst of **13**-methyl ester introduced two hydrogens in the pentenyl side chain as shown by the key ion at *m/z* 170 ($[M-C_5H_{10}]^+$) in the mass spectrum. The **12**-methyl ester showed a similar fragmentation pattern as described for [^{18}O]-**12**-methyl ester [8] with the key fragments at $[M-(CH_2)_3COOMe]^+$ (*n*-butyric acid side chain), $[M-C_5H_8]^+$ (pentenyl side chain) and the typical base peak at *m/z* 83. The facile chemical isomerization of **12** to **14** confirmed a *cis*-configuration of both side chains. Compound **12** is dextrorotatory, therefore, it has (1*S*, 2*S*)-configuration [7].

The presence of **9** in the fungus *Botryodiplodia theobromae* is understandable by its biogenetic formation from 12-oxo-phytodienoic acid via β-oxidation without elimination of the ring double bond [8, 9]. Substance **12** is already known to be a precursor of 7-*iso*-jasmonic acid (**1**) [8]. The structure of the new compound **11** suggests, that enzymes of jasmonic acid biosynthesis might be able to convert also a longer unsaturated fatty acid to give this jasmonic acid-like substance, as shown in other examples [10, 11]. Substance **2**, probably formed from **1** by fungal enzymes, has already been found in flower oils of *Jasminum grandiflorum* [12].

EXPERIMENTAL

Chromatographic methods. TLC (silica gel GF_{254}): (*a*) $CHCl_3$–MeOH–HOAc (140:20:1); (*b*) *n*-hexane–EtOAc–(60:40:1); detection by anisaldehyde reagent and heating for 5–10 min at 120° [13]; prep. TLC (silanized silica gel, RP2):(c) C_6H_6–Me_2CO (17:13). CC: (600 × 20 mm) on silanized silica gel prepared by treatment of silica gel with TMCS in C_6H_6; elution with a stepwise gradient of EtOAc in $CHCl_3$. HPLC (method *a*): LiChroprep RP 8 (30–63 μm, 310 × 25 mm), elution with MeOH–0.1% H_3PO_4 in H_2O (3:2), flow rate 3 ml/min, UV detector at 228 nm; (method *b*): Polyol RP 18 (250 × 4.6 mm), elution with MeOH–0.1% H_3PO_4 in H_2O (1:1), flow rate 1 ml/min, UV-detector at 228 nm.

Fermentation. The fungus *Botryodiplodia theobromae* Pat. (strain D7/2, isolated from Cuban oranges, *Citrus sinensis* Osbeck cv. Valencia) was pre-cultured on malt agar. The mycelium was homogenized in H_2O and used for inoculation of the production medium. The fungus was grown in surface culture for 7 days at 30° in a flask (400 ml) containing 100 ml of the following medium: 30 g sucrose, 5 g soya flour, 15 ml corn steep liquor, 10 ml of mineral salt soln, diluted to 1 l with H_2O and to pH 5.4–5.6 with 1 M NaOH and sterilized at 120° for 20 min. The mineral salt soln used contained 0.5 g KH_2PO_4, 0.3 g $MgSO_4 \cdot 7\,H_2O$, 10 mg $FeSO_4 \cdot 7\,H_2O$, and 8.8 mg $ZnSO_4 \cdot 7\,H_2O$/l.

Isolation procedure. The contents from the 100 culture flasks were lyophilized and extracted with EtOAc (3 × 1.5 l). Acidic compounds were sepd by extraction into satd $NaHCO_3$ soln (3 × 150 ml). Neutral compounds (containing **2**) remained in the EtOAc layer. Re-extraction of the aq. phase with $CHCl_3$ (3 × 150 ml) after acidification to pH 3.5 with 4 M HCl gave a $CHCl_3$ extract which was dried (Na_2SO_4) and evapd. The residue was divided into three parts and each of it purified by CC. Fractions eluted with $CHCl_3$–EtOAc (9:1) gave a crude mixture which on prep. HPLC (method *a*) gave two highly enriched fractions of **9** (R_t: 66–73 min) and **11** + **12** (R_t: 96–125 min). Fractions were extracted with $CHCl_3$ after dilution with H_2O and further separated by HPLC (method *b*) giving:

(+)-4,5-*Didehydro*-7-*iso*-*jasmonic acid* [(1*R*, 2*S*)-(+)-3-*oxo*-2-(2*Z*-*pentenyl*)*cyclopent*-4-en-1-*yl*-acetic acid] (**9**). 1.5 mg; R_t in HPLC (method *b*): 9.2 min; R_f in TLC system *a* (0.20) and *b* (0.42); $[\alpha]_D^{25}$ dextrorotatory; MS (80 eV) *m/z* (rel. int.) of **9**-methyl ester: 222 $[M]^+$ (21), 193 $[M-C_2H_5]^+$ (17), 191 $[M-OMe]^+$ (11), 167 (15), 154 $[M-C_5H_8]^+$ (81), 149 $[M-(CH_2)COOMe]^+$ (18), 133 (21), 119 (16), 107 (21), 95 $[C_6H_7O]^+$ (100); ^{1}H NMR, IR of **9**-methyl ester identical with the data published [12].

(1*R*, 2*S*)-(+)-*Oxo*-2-(2*Z*-*pentenyl*)*cyclopent*-1-yl-*propionic acid* (**11**). 0.8 mg; R_t in HPLC (method *b*): 11.7 min; R_f in TLC systems *a* (0.25) and *b* (0.50); $[\alpha]_D^{25}$ + 55° (MeOH; *c* 0.8); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1734, 1650; ^{1}H NMR (200.13 MHz, $CDCl_3$, TMS as internal standard): δ 0.93 (3H, *t*, J_{AB} = 7.3 Hz, –Me), 1.73–2.73 (14H, *m*), 5.23 (1H, *dt*, J_{AB} = 10.5 Hz, J_{AX} = 7.0 Hz, =CH–), 5,45 (1H, *dt*, J_{AB} = 10.5 Hz, J_{AX} = 7.0 Hz, =CH–); MS (80 eV) *m/z* (rel. int.) of **11**-methyl ester: 238 $[M]^+$ (24), 220 $[M-H_2O]^+$ (16), 207 $[M-OMe]^+$ (12), 191 (18), 170 $[M-C_5H_8]^+$ (21), 164 (8), 165 $[M-(CH_2)COOMe]^+$ (7), 151 $[M-(CH_2)_2COOMe]^+$ (75), 133 (13), 121 (9), 109 (40), 97 (67), 83 $[C_5H_7O]^+$ (100).

(1*S*,2*S*)-(+)-3-*Oxo*-2-(2*Z*-*pentenyl*)*cyclopent*-1-yl-*butyric acid* (**12**). 0.9 mg; R_t in HPLC (method *b*): 12.5 min; R_f in TLC systems *a* (0.27) and *b* (0.53); $[\alpha]_D^{25}$ + 48°; IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3000, 1730, 1652; ^{1}H NMR (200.13 MHz, $CDCl_3$, TMS as internal standard): δ 0.95 (3H, *t*, *J* = 7.2 Hz, –Me), 1.70–2.75 (16H, *m*), 5.23 (1H, *dt*, J_{AB} = 10.5 Hz, J_{AX} = 7.0 Hz, =CH–); 5.44 (1H, *dt*, J_{AB} = 10.5 Hz, J_{AX} = 7.0 Hz, =CH–); MS (80 eV) *m/z* (rel. int.) of **12**-methyl ester: 252 $[M]^+$ (15), 234 (12), 221 $[M-OMe]^+$ (5), 196 (10), 184 $[M-C_5H_8]^+$ (12), 151 $[M-(CH_2)_3COOMe]^+$ (61), 133 (27), 124 (13), 109 (27), 95 (37), 83 $[C_5H_7O]^+$ (100).

The neutral EtOAc extract was dried (Na_2SO_4), evapd and sepd on CC. Fractions eluted with $CHCl_3$–EtOAc (95:5) were separated by prep. TLC with system *c*. Substances between R_f: 0.60–0.73 were recovered which on HPLC (method 2) gave:

Ethyl (+)-7-*iso*-*jasmonic acid* [*ethyl* (1*R*, 2*S*)-(+)-3-*oxo*-2-(2*Z*-*pentenyl*)*cyclopent*-1-*yl*-*acetate*] (**2**). 1.1 mg, R_t in HPLC (method 2): 14.5 min; R_f in TLC system *a* (0.91) and *b* (0.68), $[\alpha]_D^{20}$ dextrorotatory; MS (80 eV) *m/z* (rel. int.) 238 $[M]^+$ (23), 220 $[M-H_2O]^+$ (6), 209 $[M-C_2H_5]^+$ (4), 193 $[M-OC_2H_5]^+$ (17), 191 $[M-C_2H_5-H_2O]^+$ (12), 170 $[M-C_5H_8]^+$ (16), 151 $[M-CH_2COOC_2H_5]^+$ (43), 133 (23), 109 (34), 95 (43), 93 (39), 83 $[C_5H_7O]^+$ (100), 79 (35); ^{1}H NMR, IR identical with published data [12] and authentic (–)-**6** prepared from (–)-**5**, EtOH and H_2SO_4.

Catalytic hydrogenation of **9**-methyl ester and **13**-*methyl ester.* Adams catalyst (2 mg) in 5 ml EtOH were satd with H_2 and 500 μg **9**-methyl ester or **13**-methyl ester added and after 15 min reduction products were recovered giving, in the case of **9**-methyl ester, 9, 10-dihydro-**1**-methyl ester [2, 4] and 9,10-dihydro-**5**-methyl ester [2, 5] or 10,11-dihydro-**13**-methyl ester. MS (80 eV) *m/z* (rel. int.) of the 10,11-dihydro-**13**-methyl ester: 240 $[M]^+$ (5), 207 (7), 170 $[M-C_5H_{10}]^+$ (27), 153 $[M-(CH_2)_2COOMe]^+$ (43), 109 (9), 96 (32), 83 $[C_5H_7O]^+$ (100).

Reduction of **9** *with* $NaBH_4$. Compound **9** (200 μg) was dissolved in a soln of 2 mg $NaHCO_3$ in 1 ml H_2O and treated with 2 mg $NaBH_4$ for 30 min at room temp. After acidification to pH 3 with 1 M HCl, extraction with $CHCl_3$, drying of the $CHCl_3$ extract with Na_2SO_4 and evapn of the solvent, TLC using solvent system *b* yielded 40% **3**, 40% **4**, 10% **7** and 10% **8** [3].

Isomerization of **2**, **9**, **11** *and* **12**. Compounds **2**, **9**, **11** and **12** (50 μg of each) were treated with 100 μl 1 M KOH. After 1 hr at 60°, 150 μl 1 M HCl was added and the solns extracted with $CHCl_3$. TLC with solvent system *a* yielded **5** (R_f: 0.28) [6], **10** (0.24) [12], **13** (0.30), and **14** (0.32), respectively. The MS of **13** was identical with that of **11**, and of **14** with that of **12**.

Analytical methods. Methyl esters were prepared by treatment of the carboxylic acids with ethereal CH_2N_2 and analysed by

GC-MS under the following conditions: 80 eV mass spectrometer, glass column (1.80 m × 2 mm) containing 10% EG SS-X on Gas Chrom P (125–150 μm), column temp. 175°, He 15 ml/min, R_t (min) of the methyl esters: **1** 12.6, **3** 15.8, **4** 13.8, **5** 10.3, **7** 11.9, **8** 11.8; steel column (1.50 m × 2 mm) containing 3% OV-225 on Gas Chrom Q (100–120 mesh), column temp. 170° (6 min)–200° (4°/min); He 17 ml/min, R_t (min) of the methyl esters: **1** 4.2, **5** 3.5, **9** 4.6, **10** 4.0, **11** 7.0, **12** 9.4, **13** 6.1, **14** 8.3, 9,10-dihydro-**1** 3.1, 9,10-dihydro-**5** 3.4; R_t (min): **6** 4.0, **2** 4.6.

Acknowledgements—The authors are grateful to Mrs M. Krohn for technical assistance, Mrs C. Kuhnt for combined GC-MS analysis and Mrs G. Geiseler for HPLC separations.

REFERENCES

1. Aldridge, D. C., Galt, S., Giles, D. and Turner, W. B. (1971) *J. Chem. Soc. C* 1623.
2. Miersch, O., Preiss, A., Sembdner, G. and Schreiber, K. (1987) *Phytochemistry* **26**, 1037.
3. Miersch, O., Meyer, A., Vorkefeld, S. and Sembdner, G. (1986) *J. Plant Growth Regul.* **5**, 91.
4. Kitahara, T., Hamaguchi, K., Warita, Y., Tagaki, Y. and Mori, K. (1986) *Agric. Biol. Chem.* **50**, 1867.
5. Cross, B. E. and Webster, G. R. B. (1970) *J. Chem. Soc. C* 1839.
6. Dathe, W., Rönsch, H., Preiss, A., Schade, W., Sembdner, G. and Schreiber, K. (1981) *Planta* **155**, 530.
7. Nishida, R., Acree, T. E. and Fukami, H. (1985) *Agric. Biol. Chem.* **49**, 769.
8. Vick, B. A. and Zimmerman, D. C. (1984) *Plant Physiol.* **75**, 458.
9. Vick, B. A. and Zimmerman, D. C. (1986) *Plant Physiol.* **80**, 202.
10. Vick, B. A. and Zimmerman, D. C. (1979) *Plant Physiol.* **63**, 490.
11. Corey, E. J. and Matsuda, S. P. (1987) *Tetrahedron Letters* **28**, 4247.
12. Kaiser, R. and Lamparsky, D. (1974) *Tetrahedron Letters* 3413.
13. Stahl, E. and Glatz, A. (1982) *J. Chromatogr.* **243**, 139.