54. Metabolic Products of Microorganisms

Part 269[1])

5-Phenylpentadienoic-Acid Derivatives from *Streptomyces* sp.

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Two new phenylpentadienamides isolated from the culture filtrate of *Streptomyces* sp. were assigned the structures 5-(4-aminophenyl)penta-2,4-dienamide (**2**) and N^2-[5-(4-aminophenyl)penta-2,4-dienoyl]-L-glutamine (**3**). In addition, 5-(4-aminophenyl)penta-2,4-dienoic acid (**1**) has been isolated, and its spectroscopic characteristics are reported for the first time. Compounds **1–3** exist in both the (2*E*,4*E*)- and (2*E*,4*Z*)-configurations. Electrospray and tandem MS, and HPLC/MS proved to be particularly suitable for the characterization of these metabolites.

Introduction. – As part of our chemical screening program, we observed that *Streptomyces* sp. Tü 3946 excreted into the culture broth a series of light yellow metabolites. These compounds appeared as orange spots, when TLC plates were sprayed with *Ehrlich* reagent [2]. Here, we report on the isolation and structure elucidation of three of these metabolites. Electrospray MS, which was introduced as a highly sensitive and soft ionization technique for the mass-spectrometric analysis of polar, non-volatile, and thermolabile molecules [3], was used for the characterization of these substances and is described in detail.

Results. – The metabolites were separated from the culture filtrate of *Streptomyces* sp. Tü 3946 by adsorption on *Amberlite XAD 16*. Subsequent fractionation by a combination of gel filtration on *Sephadex LH-20* and reversed-phase chromatography on *C-18* provided compounds **1–3** in pure form (see *Exper. Part*).

HPLC coupled with electrospray MS and diode-array detection revealed that **1–3** were a mixture of two isomers each. Both isomers showed identical fragmentation patterns but differed in their UV-absorption maxima by *ca.* 10 nm (*e.g.* λ_{max} = 305 and 294 nm for **1**). Both isomers were preparatively separated by HPLC. However, on evaporation of the solvent (temperature < 40°), mixtures once again resulted from each one. The isomeric mixtures **1–3** were, therefore, used for the structure elucidation.

Coumpound **1** (mol. wt. 189) was assigned the molecular formula $C_{11}H_{11}NO_2$ by HR-EI-MS. The NMR data (see *Tables 1* and *2*) revealed a penta-2,4-dienoic-acid moiety bonded to a *p*-substituted Ph ring. 1D-COSY [4],

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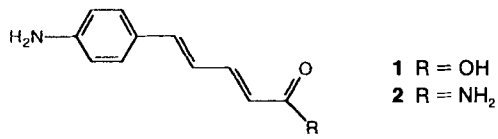
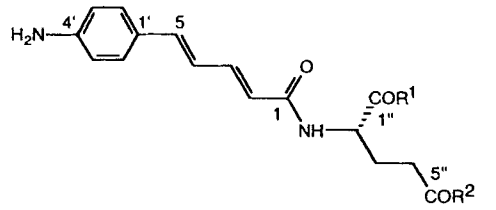


1 R = OH
2 R = NH_2



	R'	R²
3	OH	NH_2
4	NH_2	OH
5	CH_3O	NH_2

HMQC [5], and HMBC [6] experiments allowed complete assignment of ^{1}H- and ^{13}C-NMR signals. Both isomers were shown to differ only in the configuration at the C(4)=C(5) bond. The coupling constant $J(4,5)$ was found to be either 15.4 Hz ((*E*)-configuration) or 11.7 Hz ((*Z*)-configuration; δ(H–C(5)) 6.83 and 6.67 ppm, respectively). On the other hand, $J(2,3)$ (15.1 Hz) was identical in both isomers (δ(H–C(2)) 5.85 and 5.93 ppm, respectively). Corresponding attribution of H–C(2) and H–C(5) was confirmed by a heteronuclear long-range coupling between H–C(2) and C(1) detected in the HMBC spectrum. Thus, compound **1** constitutes a mixture of (2*E*,4*E*)- and (2*E*,4*Z*)-isomers of 5-(4-aminophenyl)penta-2,4-dienoic acid. The NMR and EI-MS data are in very good agreement with those reported for (2*E*,4*E*)-5-(4-hydroxyphenyl)penta-2,4-dienoic acid (avenalumic acid) [7].

The molecular formula of compound **2** (mol. wt. 188) was established as $C_{11}H_{12}N_2O$ by HR-EI-MS. The ^{1}H- and ^{13}C-NMR data (see *Tables 1* and *2*) were very similar to those of **1**. The structure was established to be 5-(4-aminophenyl)penta-2,4-dienamide. As in **1**, the C(4)=C(5) bond exists in either the (*Z*)- or (*E*)-configuration.

Table 1. *^{1}H-NMR Data* (CD_3OD, 400.1 MHz) *of Compounds* **1**–**3**

	1		**2**		**3**	
H-Atom	(2*E*,4*E*)[a])	(2*E*,4*Z*)	(2*E*,4*E*)	(2*E*,4*Z*)	(2*E*,4*E*)	(2*E*,4*Z*)
H–C(2)	5.85 (*d*, *J* = 15.1)	5.93 (*d*, *J* = 15.1)	6.00 (*d*, *J* = 15.1)	6.09 (*d*, *J* = 15.0)	6.07 (*d*, *J* = 15.0)	6.16 (*d*, *J* = 15.0)
H–C(3)	7.40 (*dd*, *J* = 15.1, 10.5)	7.84 (*dd*, *J* = 15.1, 12.1)	7.29 (*dd*, *J* = 15.1, 10.5)	7.77 (*dd*, *J* = 15.0, 11.8)	7.26 (*dd*, *J* = 15.0, 9.7)	7.75 (*dd*, *J* = 15.0, 11.8)
H–C(4)	6.74 (*dd*, *J* = 15.4, 10.5)	6.19 (*pseudo-t*, *J* = 11.6)	6.71 (*dd*, *J* = 15.5, 10.5)	6.16 (*pseudo-t*, *J* = 11.8)	6.68 (*dd*, *J* = 15.1, 9.7)	6.14 (*pseudo-t*, *J* = 11.5)
H–C(5)	6.83 (*d*, *J* = 15.4)	6.67[b]) (*d*, *J* = 11.7)	6.79 (*d*, *J* = 15.5)	6.62 (*d*, *J* = 11.8)	6.74 (*d*, *J* = 15.1)	6.57 (*d*, *J* = 11.3)
H–C(2′/6′)	7.27 (*d*, *J* = 8.4)	7.13 (*d*, *J* = 8.4)	7.25 (*d*, *J* = 8.3)	7.15 (*d*, *J* = 8.5)	7.22 (*d*, *J* = 8.5)	7.13 (*d*, *J* = 8.5)
H–C(3′/5′)	6.65 (*d*, *J* = 8.4)	6.69 (*d*, *J* = 8.4)	6.64 (*d*, *J* = 8.3)	6.68 (*d*, *J* = 8.5)	6.64 (*d*, *J* = 8.5)	6.67 (*d*, *J* = 8.5)
H–C(2″)					4.38 (*m*)	4.38 (*m*)
H–C(3″)					1.95–2.05 (*m*); 2.15–2.25 (*m*)	1.95–2.05 (*m*); 2.15–2.25 (*m*)
H–C(4″)					2.4–2.25 (*m*)	2.4–2.25 (*m*)

[a]) Configuration of the pentadienoyl moiety. [b]) Obscured; signal observed in a 1D-COSY experiment [4] by selective irradiation of H–C(4) *via* a 100-ms Eburp-shaped pulse.

Table 2. *^{13}C-NMR Data* (CD_3OD, 100.6 MHz) *of Compounds* **1**–**3**. Assignments based on HMQC spectra.

	1		**2**		**3**	
C-Atom	(2*E*,4*E*)[a])	(2*E*,4*Z*)	(2*E*,4*E*)	(2*E*,4*Z*)	(2*E*,4*E*)	(2*E*,4*Z*)
C(1)	171.3	171.2	171.8	171.6	168.6	168.4
C(2)	119.4	122.9	121.5	124.8	122.5	125.7[b])
C(3)	147.8	143.0	144.2	139.7	142.9	138.5
C(4)	122.8	124.6	123.0	124.8	123.2	125.0[b])
C(5)	142.9	139.6	141.9	138.7	141.2	138.0
C(1′)	126.9	127.3	127.2	127.5	127.2	127.6
C(2′/6′)	129.9	131.8	129.6	131.8	129.5	131.7
C(3′/5′)	116.0	115.9	116.0	115.9	116.0	115.8
C(4′)	150.8	149.8	150.5	149.6	150.2	149.3
C(1″)					178.4[c])	178.4[c])
C(2″)					55.8	55.8
C(3″)					30.2	30.2
C(4″)					33.1	33.1
C(5″)					178.4[c])	178.4[c])

[a]) Configuration of the pentadienoyl moiety. [b]) Values interchangeable although these attributions are preferred. [c]) Broad signal, unresolved.

The UV data of **3** suggested that, as **1** and **2**, a phenylpentadienoyl moiety was present. Acidic hydrolysis of **3** (6N HCl) and amino-acid analysis by GLC on a chiral phase [8] revealed the presence of L-glutamic acid. The electrospray-MS of **3** showed pseudomolecular ions at *m/z* 318 ($[M + H]^+$) and 635 ($[2M + H]^+$). In addition, an intense fragment ion was observed at *m/z* 172 which was attributed to the acylium ion corresponding to the phenylpentadienoyl moiety of the molecule. The cleavage of the amide bond also occurred by collision-induced dissociation (CID) of the $[M + H]^+$ ion with Ar (see *Fig.*). The reaction of **3** with CH_2N_2 resulted mainly in a monomethylated product **5**, as indicated by an increase of the mol. wt. of 14 u, which demonstrated the presence of an acidic group in the molecule. CID of the $[M + H]^+$ ion of this product proved that the acyl part remained unchanged, since the fragment ion at *m/z* 172 was still the dominant ion in the daughter-ion mass spectrum. All data obtained so far were in agreement with a (4-aminophenyl)pentadienoyl derivative of an amide of glutamic acid. To localize the amide function, compounds **3** and **4** were prepared by condensation of **1** with glutamine or isoglutamine (4,5-diamino-5-oxopentanoic acid), respectively. Both synthetic compounds had a mol. wt. of 317

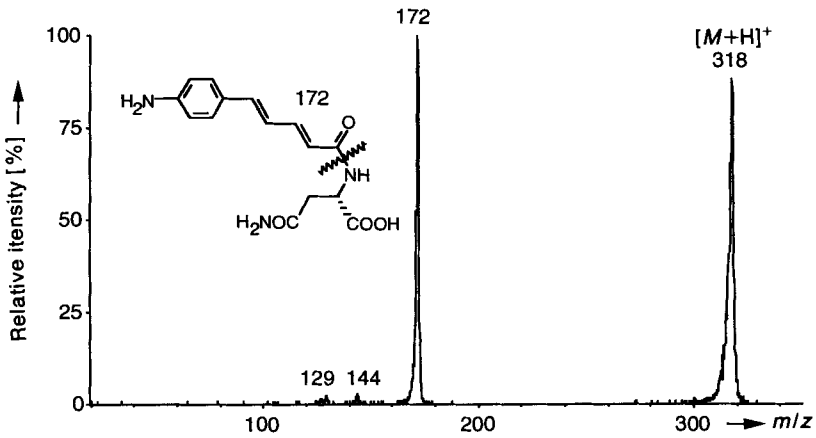


Figure. *Daughter-ion mass spectrum of* **3** (CID of $[M + H]^+$, *m/z* 318)

determined by electrospray MS and afforded a dominant fragment ion at m/z 172 in the daughter ions MS of the $[M + H]^+$ peak. While synthetic **3** proved to be identical with the natural compound, **4** clearly differed from the latter on TLC and HPLC analyses. Thus, compound **3** is N^2-[5-(4-aminophenyl)penta-2,4-dienoyl]-L-glutamine. The C(4)=C(5) bond exists in either the (*E*)- or (*Z*)-configuration.

Discussion. – Compounds **1**–**3** were not detected in the mycelium. It is not clear, whether (2*E*,4*E*)- and (2*E*,4*Z*)-isomers were both synthesized by the microbial strain, or if isomerization occurred during the fermentation and isolation procedure. Interestingly, avenalumic acid undergoes isomerization on exposure to daylight [7]. The amides **2** and **3** have so far not been described in the literature. The chemical synthesis of **1** has been reported already in the beginning of this century [9], but no spectroscopic data have been available up to now. Compound **1** has been obtained for the first time from a biological source. 5-Phenylpenta-2,4-dienoic-acid derivatives are rather unusual natural products. 5-Phenylpenta-2,4-dienoic acid itself has been detected in the bud exudate of *Populus* species [10] [11]. Some *p*-hydroxy derivatives have also been found in plants: psilotic acid has been isolated from *Psilotum nudum* [12], while several avenalumic acid conjugates were obtained from oat groats and hulls [7]. On the other hand, there is no report about the production of phenyl-pentadienoic-acid derivatives by microorganisms. Compounds **1**–**3** proved to be inactive against Gram-positive and Gram-negative bacteria in our testing systems.

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Experimental Part

General. H-L-Gln-O^{t}Bu · HCl and H-L-Glu(O^{t}Bu)-NH_2 · HCl were purchased from *Bachem Biochemica*. Prep. low-pressure liquid chromatography (LPLC): *Lobar C-18* column (40–63 μm; i.d. 2.5 × 27 cm; *Merck*) equipped with a *FR-30* HPLC pump (*Knauer*). Prep. HPLC: *Nucleosil 100 C-18* column (10 μm, i.d. 250 × 16 mm) equipped with a precolumn (i.d. 30 × 16 mm), 2 *Sepapress HPP 200/100* high-performance pumps (*Kronwald*) and a *Sepacon GCU-311* gradient control unit (*Kronwald*). TLC: silica-gel-precoated Al sheet (*Macherey-Nagel*), BuOH/EtOH/H_2O 6:2:2 (eluent *1*). GLC: *GC-Sichromat 1* (*Siemens*) equipped with a N-selective detector and an *Autoderivat 100* derivatization system (*CAT*, Tübingen, Germany). M.p.: given for the mixture of stereoisomers and are uncorrected. $[\alpha]_D$: *Perkin-Elmer 241* polarimeter; 0.1-dm cell. IR: *Perkin-Elmer 281 B* infrared spectrophotometer. NMR: High-resolution spectra were obtained on a *Bruker AMX 400* spectrometer interfaced to a *X32* computer and equipped with an inverse triple resonance probe; 50 mM soln. in CD_3OD were used; chemical shifts were referenced to the solvent peak (δ(H) 3.30 ppm; δ(C) 49.0 ppm); assignments by the use of 1D-COSY [4], heteronuclear multiple quantum coherence (HMQC) [5] and heteronuclear multiple bond correlation (HMBC) [6] experiments.

Microbial Strain. Streptomyces sp. Tü 3946 was isolated from a soil sample collected in 1990 in New Zealand. The strain builds a gray spore mass on yeast extract agar. The spore surface is spiny. Melanin is not produced on peptone/yeast extract/iron agar.

Production and Isolation. The strain Tü 3946 was cultivated in 500-ml *Erlenmeyer* flasks with four baffles containing each 150 ml of a medium consisting of decreased soybean meal (2%), starch (1%), glycerol (1%), and NaBr (0.03%; pH 7.75). Each flask was inoculated with 5 ml of a 36-h old preculture (medium: soybean meal (2%), mannitol (2%), pH 7.5). The fermentation was carried out on a rotary shaker for 4 d at 27°. After filtration of the culture broth (5 l), the culture filtrate was passed through a *Amberlite XAD 16* column (500 ml), and the

compounds were then eluted with MeOH (2 l). After evaporation, a 8.6-g portion of the residue (9.1 g) was fractionated on a *Sephadex LH-20* column (i.d. 61 × 5 cm) with MeOH/H_2O 1:1. Fourteen fractions were collected (I–XIV). Compound **1** was purified from *Fraction VII* (74 mg) by LPLC on *RP-18* with MeOH/H_2O 4:6; compounds **2** and **3** were obtained following the same procedure from *Fractions XIII* (82 mg) and *V* (930 mg), resp., with MeOH/H_2O 2:8 and 3:7, resp. Final purification of compounds **1** (3 mg), **2** (12 mg), and **3** (117 mg) was achieved by prep. HPLC on *RP-18* with following eluents (flow rate 20 ml/min): *1.* MeOH/0.05% aq. TFA 1:9→1:0 in 30 min; *2.* MeCN/H_2O 0:1→7:3 in 10 min; *3.* MeOH/H_2O 1:9→1:1 in 15 min.

Mass Spectrometry. EI-MS: *TSQ 70* spectrometer (*Finnigan MAT*). HR-EI-MS: Modified *MAT 711A* spectrometer (*AMD INTECTRA*). Electrospray MS and tandem MS: Measurements were performed on a *Sciex API III* triple-quadrupole mass spectrometer with mass range of 2400 Da equipped with a nebulizer-assisted electrospray ('ion spray') ion source (*Sciex*, Thornhill, Ontario, Canada). The mass spectrometer was operated in positive-ion mode under conditions of unit mass resolution for all determinations. The accuracy of mass determination was ± 0.1 u. Profile spectra were obtained by acquiring data points every 0.1 Da. Electrospray voltage was +4.9 kV, orifice voltage was +80 V. Collision-induced dissociation experiments (CID, daughter-ion scans) were performed using Ar at a target gas thickness of *ca.* 5×10^{14} atoms cm^{-2} with collision energies of *ca.* 40 eV. Daughter ions were obtained by CID of the molecular ion $[M + H]^+$. The compounds were dissolved in MeOH/1% aq. HCOOH and were analyzed by electrospray MS and tandem MS (MS/MS) either directly or on-line in combination with a narrow-bore HPLC-system (*Applied Biosystem 140A*). For direct injection, the soln. was introduced into the electrospray source at a constant flow rate of 5 µl/min with a medical syringe infusion pump (*Harvard Apparatus*, modell *22*, Southnatick, USA) in combination with a microliter syringe (100 µl, *Hamilton, # 1710*, USA).

HPLC/MS. Analyses were performed on a *Nucleosil C-18* narrow-bore column (5 µm; 100 × 2 mm) equipped with a precolumn (10 × 2 mm). The column was connected with the electrospray interface *via* a fused capillary (length 30 cm; 100 µm i.d.). A gradient of aq. MeCN, containing TFA (0.1%), was used as eluent (MeCN/H_2O 0:1→1:1 in 10 min, flow-rate 200 µl/min; 40 µl/min into MS); detection by electrospray MS.

HPLC/UV. HP 1090M Liquid chromatograph equipped with a diode array detection system and a work station (*Hewlett-Packard*). Analyses were performed on a *Nucleosil C-18* column (5 µm; i.d. 125 × 4.6 mm) equipped with a precolumn (i.d. 20 × 4.6 mm). A gradient of aq. MeCN was used as eluent (MeCN/0.1% aq. H_3PO_4 0:1→1:1 in 10 min, flow-rate 2 ml/min).

Amino-Acid Analysis. Compound **3** (0.1 mg) was dissolved in 6N HCl and hydrolyzed for 18 h at 110°. Derivatization was performed directly before the analysis by successive treatment with 3N HCl/PrOH (110°, 30 min) and $(CF_3CO)_2O/CF_3COOEt$ (140°, 10 min). After evaporation of the reagents, the sample was dissolved in toluene/Me_2CO 3:1 and analyzed by GLC on a *Chirasil-Val* glass capillary column (20 m × 0.3 mm) [8]; the oven temp. programme was 80° for 3 min, then 80° to 190° at 4° min^{-1}. L-Glutamic acid was identified by comparison with a standard.

5-(4-Aminophenyl)penta-2,4-dienoic acid (**1**, $C_{11}H_{11}NO_2$). Orange-to-brown solid. M.p. 178°. TLC (SiO_2, eluent *1*): R_f 0.56. IR (KBr): 3360, 3200, 1675, 1590, 1510, 1295, 1265, 995. HPLC/UV ((2*E*,4*E*)-isomer; r.t., 4.5 min): 305, 230. HPLC/UV ((2*E*,4*Z*)-isomer; r.t., 4.9 min): 294, 224. Electrospray-MS: 190 ($[M + H]^+$). EI-MS (70 eV): 189 (19), 144 (100), 127 (18), 155 (11). HR-EI-MS ($[M]^+$): calc. 189.0800, found 189.0795.

5-(4-Aminophenyl)penta-2,4-dienamide (**2**, $C_{11}H_{12}N_2O$). Yellow solid. M.p. 205°. TLC (SiO_2, eluent *1*): R_f 0.63. IR (KBr): 3320, 3200, 1650, 1580, 1510, 1385, 1290, 990. HPLC/UV ((2*E*,4*E*)-isomer; r.t., 3.4 min): 303, 228. HPLC/UV ((2*E*,4*Z*)-isomer; r.t. 3,7 min): 292, 224. Electrospray-MS: 189 ($[M + H]^+$). EI-MS (70 eV): 188 (5), 144 (100), 143 (60), 127 (30), 115 (24). HR-EI-MS ($[M]^+$): calc. 188.0949, found 188.0944.

N^2-*[5-(4-Aminophenyl)penta-2,4-dienoyl]*-L-*glutamine* (**3**, $C_{16}H_{19}N_3O_4$). Yellow-to-orange solid. M.p. 250° (dec.). $[\alpha]_D^{30} = -4.9$ ($c = 0.205$, DMSO). TLC (SiO_3, eluent *1*): R_f 0.36. IR (KBr): 3350, 1650, 1585, 1505, 1395, 1285, 995. HPLC/UV ((2*E*,4*E*)-isomer; r.t., 3.5 min): 306, 230. HPLC/UV ((2*E*,4*Z*)-isomer; r.t., 3.7 min): 295, 225. Electrospray-MS: 318 ($[M + H]^+$). Tandem-MS (electrospray-MS, CID of $[M + H]^+$): 318, 172.

N^2-*[5-(4-Aminophenyl)penta-2,4-dienoyl]*-L-*glutamine Methyl Ester* (**5**, $C_{17}H_{21}N_3O_4$). A soln. (1 ml) of CH_2N_2 in Et_2O was added to a soln. of **3** (17 mg) in BuOH/MeOH 2:1 (1.5 ml). After stirring for 15 h at r.t., the solvent was evaporated and the crude product purified by gel filtration on *Sephadex LH-20* with MeOH/H_2O 1:1. Final purification by HPLC on *RP-18* with MeCN/H_2O (2:8→8:2 in 30 min) provided 3 mg of **4**. Yellow solid. ^{1}H-NMR (CD_3OD, (2*E*,4*E*)-isomer): 7.30 (*dd*, $J = 15.0, 10.4$, H–C(3)); 7.25 (*d*, $J = 8.6$, H–C(2′), H–C(6′)); 6.80 (*d*, $J = 15.5$, H–C(5)); 6.72 (*dd*, $J = 15.5, 10.4$, H–C(4)); 6.65 (*d*, $J = 8.4$, H–C(3′), H–C(5′)); 6.05 (*d*, $J = 15.0$, H–C(2)); 4.50 (*dd*, $J = 8.8, 5.3$, H–C(2″)); 3.73 (*s*, MeO); 2.35–2.30 (*m*, 2 H–C(4″)); 2.25–2.15, 2.05–1.95 (2*m*, 2 H–C(3″)). Electrospray-MS: 332 ($[M + H]^+$). Tandem-MS (electrospray-MS, CID of $[M + H]^+$): 332, 172.

Preparation of **3** *and* **4** *from* **1**. *Compound* **3**. *N,N'*-diisopropylcarbodiimid (8.3 µl, 53 µmol), 1-hydroxy-1*H*-benzotriazol (7.2 mg, 53 µmol), Et(i-Pr)$_2$N (18 µl, 105 µmol), and 4-(dimethylamino)pyridin (0.7 mg, 5.7 µmol) were dissolved in 1 ml of DMF. This soln. (0.1 ml) was added to a soln. of **1** (1 mg, 5.3 µmol) and H-L-Gln-O(*t*-Bu)·HCl (1.4 mg, 5.8 µmol) in DMF (0.1 ml). The mixture was kept at 60° for 15 h. After evaporation of the solvent, the dry residue was treated with 0.4 ml of TFA for 30 min. Purification by HPLC on *RP-18* with MeCN/0.1% aq. TFA (0:1→2:8 in 10 min) provided 0.6 mg of **3** identical (DC, HPLC, HPLC/MS and tandem-MS) with the natural compound. *Compound* **4**. N^2-*[5-(4-Aminophenyl)penta-2,4-dienoyl]-*L-*isoglutamine* (**4**) was prepared from **1** (1 mg) and H-L-Glu(O(*t*-Bu))-NH$_2$·HCl (1.4 mg) following the same procedure. Purification as described for **3** provided 0.8 mg of **4**. TLC (SiO_2, eluent *1*): R_f 0.47. Electrospray-MS: 318 ($[M + H]^+$). Tandem-MS (electrospray-MS, CID of $[M + H]^+$): 318,172.

REFERENCES

[1] M. Ritzau, S. Philips, A. Zeeck, H. Hoff, H. Zähner, 'Obscurolides, a novel class of phosphodiesterase inhibitors from *Streptomyces*. II. Minor components belonging to the obscurolide B to D series', *J. Antibiot.* **1993,** *46,* 1625.
[2] E. Stahl, 'Dünnschicht-Chromatographie', 2nd edn., Springer-Verlag, Berlin–Heidelberg–New York, 1967, p. 825.
[3] A. P. Bruins, T. R. Covey, J. D. Henion, *Anal. Chem.* **1987,** *59,* 2642.
[4] H. Kessler, H. Oschkinat, C. Griesinger, W. Bermel, *J. Magn. Reson.* **1986,** *70,* 106.
[5] L. Müller, *J. Am. Chem. Soc.* **1979,** *101,* 4481.
[6] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986,** *108,* 2093.
[7] F. W. Collins, D. C. McLachlan, B. A. Blackwell, *Cereal Chem.* **1992,** *68,* 184.
[8] H. Frank, G. Nicholson, E. Bayer, *J. Chromatogr. Sci.* **1977,** *15,* 174.
[9] H. Fecht, *Ber. Dtsch. Chem. Ges.* **1907,** *40,* 3891.
[10] W. Greenaway, F. R. Whatley, *Phytochemistry* **1990,** *29,* 2551.
[11] W. Greenaway, S. English, F. R. Whatley, *Z. Naturforsch., C* **1990,** *45,* 931.
[12] T. Shamsuddin, A. Khan, I. Ahmad, W. Rahman, K. M. Shamsuddin, *Phytochemistry* **1985,** *24,* 2458.