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SPRUCE DIE-BACK: ISOLATION OF *p*-HYDROXYACETOPHENONE FROM DISEASED SHOOTS OF *PICEA ABIES*

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Key Word Index—*Picea abies*; Pinaceae; Norway spruce, spruce die-back; forest decline; shoots; identification; *p*-hydroxyacetophenone.

Abstract—*p*-Hydroxyacetophenone as a new growth substance exhibiting inhibitory activity was isolated from shoots of *Picea abies* damaged by die-back. Its identity was established by chromatographic and spectroscopic methods.

INTRODUCTION

In continuation of our search for the biologically active compounds present in Norway spruce damaged by spruce die-back, we detected a number of compounds differing both qualitatively and quantitatively from healthy plants [1–3]. The present investigation was directed to the identification of growth inhibitors present in the neutral diethyl ether extract of diseased shoots.

RESULTS AND DISCUSSION

About 2.7 kg of diseased *Picea abies* shoots (10 year old trees) were harvested, extracted and purified according to the procedure described [1]. The neutral diethyl ether extract was subjected to silica gel partition chromatography (32 × 2.4 cm) to give a strong phytotoxic zone (wheat and rice seedling bioassay), which was eluted with chloroform–ethyl acetate, 9:1–3:1. The phytotoxic zone was purified on DEAE-Sephadex A-25 (132 × 1.7 cm) to produce a strong phytotoxic fraction (eluted with 80% MeOH). TLC of this fraction after double developments (SS 1 and SS 2) yielded a strong biologically active fluorescent quenching band (R_f = 0.46–0.96). Re-chromatography of this band after double developments (SS 3 and SS 4) using TLC gave a strong phytotoxic zone (R_f = 0.37–0.73). This phytotoxic zone was further purified by TLC (R_f = 0.61–0.91, SS 5). The purified phytotoxic zone was subjected to silica gel partition column and the subsequent bioassay showed a main active zone (eluted with *n*-hexane–chloroform, 7:3). The main active zone was purified further by silica gel adsorption chromatography using the same gradient as described for partition chromatography. The adsorption chromatography yielded a crystalline substance, which was sparingly soluble in water and thus, purified again with repeated washings with water. The purified substance was again subjected to TLC (silanized silica gel F_{254}; SS 6, R_f = 0.05–0.35). The HPTLC (silica gel F_{254}: SS 6, R_f = 0.19) showed a single strong fluorescent spot, which gave an intense purple coloration after treatment with 5% ethanolic sulphuric acid and subsequent heating at 120° for 30 min. This spot was found to be identical with authentic *p*-hydroxyacetophenone. UV, IR, mass and ^{1}H NMR spectroscopy further confirmed the identity of the isolated compound.

This is the first identification of *p*-hydroxyacetophenone as a growth substance from the diseased shoots of *Picea abies*. In the past *p*-hydroxyacetophenone has been synthesized and is still used for the synthesis of different compounds of industrial importance. The first isolation of *p*-hydroxyacetophenone was reported from the ether soluble oil of buds of *Populus trichocarpa* [4], the plant growth regulating activity of which was not reported. At lower concentrations, *p*-hydroxyacetophenone was found to show weak gibberellin-like activity as observed in the dwarf rice seedling bioassay (Table 1). At higher concentrations, *p*-hydroxyacetophenone inhibited the growth of rice (roots) and wheat seedlings (> 809 μg/5 seedlings), and showed needle-browning and death of spruce seedlings (3 weeks old, > 81 μg/plant; 6 weeks old > 132 μg/plant, Table 1) and saplings (2 years old, > 324 μg/plant) when applied to the tip of the apical growing part. Spruce seedlings (3 weeks old) showed dramatically the needle-browning *ca* 1 hr after application-time. The application of > 1200 μg via roots

Table 1. Biological activity of *p*-hydroxyacetophenone

Growth inhibitor test				Gibberellin test			
Spruce seedling microdrop bioassay		Wheat seedling bioassay		Dwarf rice bioassay *p*-hydroxyacetophenone		Gibberellic acid 3	
Application μg/plant	Damaged seedling (%)*	Application μg/5 seedlings	Growth (%)†	Application μg/5 seedlings	Length of seedlings (cm)	Application ng/5 seedlings	Length of seedlings (cm)
0	0	0	100	0	2.3 ± 0.3	0	2.5 ± 0.2
		128	104				
66	+	256	116	81	4.6 ± 1.2	3.46	6.0 ± 0.8
		809	74				
132	30	1290	70	162	2.4 ± 0.3	34.60	9.4 ± 0.7
		2560	30				
264	100	3760	26	809	2.4 ± 0.3	346.00	11.4 ± 1.2
		5640	0‡				

*Seedlings with needle necrosis.
†Relative to control.
‡Death of seedlings.
+, Trace.

of saplings (2 years old) yielded an identical yellow–brown colouring of needles after a period of *ca* 3 weeks progressing successively from the basal to the apical part with increasing time, whereas the control plants (water) did not show such symptoms [1]. Diseased spruce needles (initial stage of disease) contain 16.1 mg *p*-hydroxyacetophenone per g dry wt, whereas healthy needles contained only 72.0 μg/g dry wt. [1].

The growth inhibitory activity as shown here is obviously dependent upon the bioassay system. The high activity of this compound in the spruce seedlings bioassay and its large accumulation in needles of affected trees would suggest the reaction of spruce trees to stress [1, 2]. The position, the function and the mode of accumulation of this compound in the diseased shoots of *Picea abies* remain open. The compound was shown *in vitro* to be an aglycone of different natural conjugates [4].

EXPERIMENTAL

Plant material. The damaged 10 cm long lateral terminal shoots (2.7 kg fr. wt) with needles showing visible yellow coloration from 10 year old trees (Forstamt Neureichenau, Bavarian Forest, Winter 1981) were collected and immediately frozen with liquid N_2 and transferred to radiation-safety vessels containing dry-ice during transport. The plant materials were freeze-dried and ground in a blender.

Extraction and purification. Ground plant material was extracted with 80% aq. MeOH. After filtration, the extract was reduced to aq. phase *in vacuo* and the aq. phase frozen. After thawing at 3° lipid material was separated by filtration through a layer of Celite-cellulose powder (1:1). The aq. filtrate was adjusted to pH 2.5 and extracted with Et_2O (4 × 1/3 vol). The Et_2O extracts were combined and partitioned with 0.1 M $NaHCO_3$ (pH 8; 1 × 1/3 vol) to give a neutral Et_2O extract. The neutral Et_2O extract was dried (Na_2SO_4) and evapd to a gum (7.62 g). The whole gum was purified by a combination of CC and TLC as described below.

CC was done by silica gel partition (32 × 2.4 cm; silica gel + 20% H_2O), DEAE-Sephadex A-25 (132 × 1.7 cm), silica gel partition column (32 × 1.6 cm; silica gel + 20% H_2O) and silica gel adsorption column (32 × 1.6 cm).

TLC was performed using the following pre-coated TLC plates (Merck) and solvent systems: SS 1—MeCOEt–Me_2CO, 1:1 (0.5 mm silica gel F_{254}); SS 2—$CHCl_3$–MeCOEt, 4:1 (0.5 mm silica gel F_{254}); SS 3—petrol (60–80°)–Me_2CO–*n*-PrOH, 16:3:1 (0.5 mm silica gel 60); SS 4—petrol (60–80°)–Me_2CO–*n*-PrOH, 7:2:1 (0.5 mm silica gel 60); SS 5—C_6H_6–Me_2CO, 7:3 (0.5 mm silica gel 60) and SS 6—$CHCl_3$ (2 mm, silanized silica gel F_{254}; HPTLC, 0.5 mm silica gel F_{254}).

p-Hydroxyacetophenone. UV λ_{max}^{MeOH} nm: 273, 217; IR ν_{max}^{KBr} cm^{-1}: 3310 (OH), 3000 (aromatic–CH), 1660 (C=O), 1575 and 1510 (aromatic C=C), 845 (1,4-disubstituted benzene); MS (70 eV) *m/z* (rel. int.): 136 $[M]^+$ (20), 121 $[M - Me]^+$ (74), 93 $[M - Ac]^+$ (22), 65 $[C_5H_5]^+$ (19); ^1H NMR (80 MHz, $CDCl_3$, TMS int. standard): δ 2.46 (3H, *s*), 6.93 (2H, *d*, H-35, *J* = 8.0 Hz), 7.93 (2H, *d*, H-26, *J* = 9.2 Hz).

Bioassay. Wheat and rice seedling bioassays were performed according to ref. [5] after some modifications. The spruce seedling microdrop bioassay was done under the following conditions: pre-germination in darkness, 5 days, 22°; growth in 50 ml 1% Agar–agar (8 × Ø 3 cm vial) for 3–6 weeks (16 klx, 16 hr, 23°, 100% rel. humidity). Samples dissolved in EtOH were applied to the apical part as 5 μl drops.

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CONFIRMATION OF THE STRUCTURE OF JEEDIFLAVANONE: A BIFLAVANONE FROM *SEMECARPUS ANACARDIUM**

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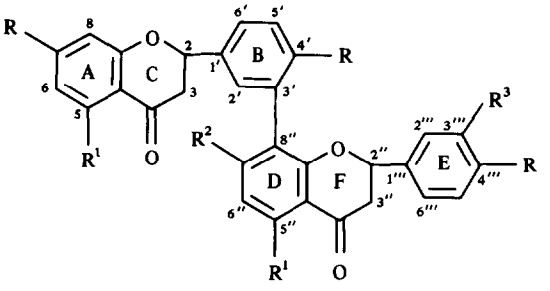
Key Word Index—*Semecarpus anacardium*; Anacardiaceae; jeediflavanone; biflavone SA5; ^{1}H NMR and mass spectra; solvent induced methoxy shifts.

Abstract—Jeediflavanone was dehydrogenated with I_2–KOAc in HOAc to the corresponding, relatively more stable biflavone designated SA5. The solvent induced methoxy shift data of SA5 heptamethyl ether confirmed the structure as well as the interflavonoid linkage of jeediflavanone.

The acetone soluble fraction of an alcoholic extract of the defatted nut shells of *Semecarpus anacardium* L. afforded a new compound jeediflavanone (**1**) [1] besides the known three biflavanones **2**–**4** [2]. The structure of jeediflavanone was elucidated mainly on the basis of ^{1}H NMR and mass spectral data and also its biflavonoid linkage at the B-3′-D-8″ position was assumed by analogy. Hence further chemical and spectral evidence is necessary to establish its structure. The present study deals with the confirmation of the structure of jeediflavanone.

Jeediflavanone (**1**) was dehydrogenated [3–5] with iodine and potassium acetate in glacial acetic acid for three hours at reflux temperature to give the corresponding stable biflavone designated SA5. It did not crystallize from common organic solvents and hence on drying in a vacuum oven at 70°, the compound appeared as a pale yellow powder from acetone, $C_{30}H_{18}O_{11}$, mp > 300°, IR (nujol): 3510–3440 (*br*, OH), 1625 (chelated flavone carbonyl), 1600 and 1590 cm^{-1} (aromatic). Compound SA5 gave a greenish-violet ferric reaction, a pink colour with magnesium–hydrochloric acid characteristic of a flavone.

The ^{1}H NMR spectrum (80 MHz, acetone-d_6, TMS as internal standard) of SA5 showed a singlet signal† at 6.50 due to two olefinic protons at C-3 and F-3″ positions. The two signals at 6.14 (*d*, J = 2.0 Hz) and 6.20 (*d*, J = 2.0 Hz) integrating each for one proton corresponded respectively to the two *meta*-coupled protons at the 6 and 8 positions of ring A. There are two low field signals at 14.24 (*s*, 1H) and 14.35 (*s*, 1H) corresponding to the two chelated hydroxylic protons at A-5 and D-5″ positions respectively [2]. Further the ^{1}H NMR spectrum indicated the presence of five non-chelated hydroxylic protons at 7.32 (*s*, 1H), 7.48 (*s*, 2H) and 7.59 (*s*, 2H) which are exchanged with D_2O and these should correspond to A-7, B-4′, D-7″ and



1 R = R¹ = R² = R³ = OH
2 R = R¹ = R³ = OH, R² = H
3 R = R¹ = R² = OH, R³ = H
4 R = R² = OH, R¹ = R³ = H

*Part 5 in the series "Partial Conversions in Biflavonoids". For Part 4 see Murthy, S. S. N., *Curr. Sci.* (communicated).

†Chemical shifts throughout this article are in δ values.