177900b.
7. Kakisawa, H., Hayashi, T. and Yamazaki, T. (1969) *Tetrahedron Letters* 301.
8. Hayashi, T., Handa, T., Ohashi, M. and Kakisawa, H. (1971) *Chem. Commun.* 541.
9. Ulubelen, A. and Miski, M. (1980) *J. Nat. Prod.* **44**, 119.
10. Saleh, M. R. I., Sabri, N. and El-Masry, S. (1978) *Egypt J. Pharm. Sci.* **19**, 313.
11. Ulubelen, A., Ozturk, S. and Isildatici, S. (1968) *J. Pharm. Sci.* 1037.
12. Schroder, H. A. (1967) *J. Chromatogr.* **30**, 537.
13. Boya, M. T. and Valverde, S. (1981) *Phytochemistry* **20**, 1367.
14. Yoshizaki, V. F., Ruedi, P. and Eugster, C. H. (1979) *Helv. Chim. Acta* **62**, 2754.
15. Al-Hazimi, H. M. G. and Miana, G. A. (1984) *J. Nat. Prod.* (submitted for publication).
16. Narayanan, C. R. and Linde, H. (1965) *Tetrahedron Letters* 3647.
17. Hodges, R., Cambie., R. C. and Joblin, K. N. (1970) *Org. Mass Spectrom.* **3**, 1473.

Phytochemistry, Vol. 23, No. 4, pp. 921–923, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

24α-METHYL-5α-CHOLEST-7-EN-3β-OL FROM SEED OIL OF *HELIANTHUS ANNUUS*

TARO MATSUMOTO, MASAYUKI NAKAGAWA and TOSHIHIRO ITOH

College of Science and Technology, Nihon University, 1-8, Kanda Surugadai, Chiyoda-ku, Tokyo, 101 Japan

(*Received* 6 *September* 1983)

Key Word Index—*Helianthus annuus*; Compositae; seeds; 24-alkylsterols; 24α-methyl-5α-cholest-7-en-3β-ol.

Abstract—24-Methyl-5α-cholest-7-en-3β-ol (24-methyllathosterol) isolated from the seed oil of *Helianthus annuus* was shown to have 24α-configuration by ^{1}H NMR spectroscopy. The stereochemistry at C-24 of some other 24-alkylsterols isolated from this plant material also was determined.

24-Methylcholest-5-en-3β-ol (24-methylcholesterol, **6**) [1–3] and 24-methyl-*trans*-22-dehydrocholesterol (**9**) [4–6] found in higher plants often consist of a mixture of epimers at C-24, i.e. campesterol (24α) and 22-dihydrobrassicasterol (24β) for **6**, and *trans*-22-dehydrocampesterol (24α) and brassicasterol (24β) for **9**. 24-Methyl-5α-cholest-7-en-3β-ol (24-methyllathosterol, **1**), a Δ^7-analog of **6**, also occurs in some higher plants including Compositae plants such as *Helianthus annuus* and *Carthamus tinctorius* [7]. However, the stereochemistry at C-24 of this sterol (**1**) from these higher plants remains undetermined to the best of our knowledge. Therefore, we have conducted here the determination of C-24 stereochemistry with the aid of 400 MHz ^{1}H NMR spectroscopy for sterol **1** and some other 24-alkylsterols isolated from the seed oil of *H. annuus* and have undertaken thorough analysis of the total sterol fraction of this plant material.

The Δ^5- (R_f = 0.33, fraction A, 291 mg) and Δ^7- (R_f = 0.31, fraction B, 50 mg) sterol fractions, separated by silica gel TLC from the unsaponifiable lipid (1.5 g) of *H. annuus* seed oil (160 g), were acetylated, and the resulting acetate fractions were subjected to silver nitrate–silica gel TLC. The acetylated fraction A gave three further fractions. Fraction A-1 from the least polar band (R_f = 0.60) was a mixture of the acetates of **6** and 24-ethylcholesterol (**7**). Fraction A-2 from the band of R_f = 0.44 was 24-ethyl-*trans*-22-dehydrocholesteryl (**8**) acetate. Fraction A-3 from several bands of R_f = 0.1–0.4 consisted of the acetates of 24-methyl-*trans*-22-dehydrocholesterol (**9**), 24*Z*-ethylidenecholesterol (isofucosterol, **11**) and 24-methylene-25-methylcholesterol (**12**). The acetylated fraction B separated into two fractions on argentation TLC. Fraction B-1 from the least polar band (R_f = 0.60) consisted of the acetates of **1** and 24-ethyllathosterol (**2**). Fraction B-2 from several bands of R_f = 0.1–0.4 was a mixture of the acetates of 24-methylenelathosterol (episterol, **3**), 24*Z*-ethylidenelathosterol (Δ^7-avenasterol, **4**) and 24-ethyl-24(25)-dehydrolathosterol (peposterol, **5**). The identifications were performed by GC and GC/MS analyses of the acetate derivatives. Acetate fraction A-2 was then hydrolysed to give free sterol **8** (mp 163–166°). Acetate fractions A-1 and B-1 were also hydrolysed and further fractionated by reverse-phase HPLC. Fraction A-1, on HPLC, yielded **6** (RR_t = 1.10, mp 145–148°) and **7** (RR_t = 1.18, mp 136–140°), and fraction B-1 gave **1** (RR_t = 1.09, mp 144–146°) and **2** (RR_t = 1.18, mp 144–148°).

In order to determine the configuration at C-24 of **1** as well as **2** and **6–8**, these sterols were subjected to 400 MHz ^{1}H NMR spectroscopy. Table 1 shows the chemical shifts of the methyl group signals of these sterols accompanied with three authentic sterols, 24β-methyllathosterol, 24α-ethyl-*trans*-22-dehydrocholesterol (stigmasterol) and its 24β-epimer (poriferasterol), for which assignments were

Table 1. Methyl group ^{1}H NMR chemical shifts (400 MHz, $CDCl_3$)* of some 24-alkylsterols

Sterol	H-18 (*s*)	H-19 (*s*)	H-21 (*d*, *J* = 6.5)	H-26 (*d*, *J* = 7)	H-27 (*d*, *J* = 7)	H-28 (*d*, *J* = 7)	H-29 (*d*, *J* = 7)
24-Methyllathosterol (**1**)	0.536	0.800	0.916	0.852	0.804	0.775	—
24β-Methyllathosterol†	0.532	0.796	0.924	0.857	0.784	0.778	—
24-Ethyllathosterol (**2**)	0.536	0.796	0.926	0.837	0.814	—	0.846
24-Methylcholesterol (**6**)	0.680	1.009	0.911‡	0.851	0.803	0.772	—
			0.919‡	0.855	0.783	0.774	—
24-Ethylcholesterol (**7**)	0.680	1.009	0.921	0.834	0.813	—	0.845
24-Ethyl-*trans*-22-dehydrocholesterol (**8**)	0.700	1.013	1.022	0.847	0.797	—	0.806
Stigmasterol (24α)†	0.699	1.012	1.021	0.846	0.797	—	0.805
Poriferasterol (24β)†	0.697	1.011	1.025	0.844	0.791	—	0.811

*Chemical shifts given in δ values from TMS; coupling constants in Hz.

†Authentic sterols.

‡Relative height of the doublets at δ0.911 and 0.919 was 7:3.

made by comparison with literature data [8–10]. Stereoisomers at C-24 of 24-methylsterols are reported to show significantly different chemical shifts for the methyl doublets of H-21 and H-27 in the high-resolution ^{1}H NMR [1,8–10]. The 24-methyllathosterol (**1**) gave H-21 and H-27 methyl doublets at δ0.916 and 0.804, respectively, which were consistent with the corresponding signals reported for the 24α-epimer [8]. Taking into consideration that the authentic 24β-epimer had quite different chemical shifts for these doublets (H-21, δ0.924; H-27, δ0.784), sterol **1** was reasonably identified as 24α-methyllathosterol. 24-Methylcholesterol (**6**) was, on the other hand, shown to be a mixture of 24α- (H-21, δ0.911; H-27, δ0.803) and 24β- (H-21, δ0.919; H-27, δ0.783) epimers. Based on the relative heights of the H-21 doublet arising from each of the two diastereoisomers, the relative ratio was estimated as 24α:24β = 7:3. The methyl group proton signals of sterol **8** were consistent with those of the authentic stigmasterol (24α) and differed enough in the chemical shifts of H-27 and H-29 methyl signals to distinguish it from its 24β-epimer (poriferasterol). Hence, sterol **8** was identified as stigmasterol. 24-Ethylsterols **2** and **7** showed almost identical ^{1}H NMR spectra with those of the authentic 24α-ethyllathosterol (22-dihydrospinasterol) and 24α-ethylcholesterol (sitosterol), respectively, described in our previous article [11]. Furthermore, the ^{1}H NMR spectra of these *H. annuus* sterols were considerably different from those of the corresponding 24β-epimers, 24β-ethyllathosterol and 24β-ethylcholesterol [11], and hence sterols **2** and **7** were regarded as the 24α-epimers, i.e. 22-dihydrospinasterol and sitosterol, respectively. The quantitative composition of the total sterol fraction of *H. annuus* seed oil was determined from the GC, argentic TLC and ^{1}H NMR data as 0.6% (**1**), 7.3% (**2**), 0.2% (**3**), 4.6% (**4**), 0.1% (**5**), 6.2% (**6**, 24α), 2.7% (**6**, 24β), 60.2%(**7**), 8.4% (**8**), 0.1% (**9**), 1.5% (**10**), 5.9% (**11**), 0.5% (**12**), and 1.7% of several unidentified components.

Thus, 24-methyllathosterol (**1**) isolated from *H. annuus* seed oil was identified as the 24α-epimer. 24-Methylcholesterol (**6**) was, on the other hand, shown to be a mixture of the 24α- and 24β-epimers, consistent with the previous observations on several other higher plants [1, 8–10]. Three 24-ethylsterols, **2**, **7** and **8**, were indicated to have the 24α-configuration. Sterol **9** remained stereochemically undetermined in this study. 24-Methylene-25-methylcholesterol (**12**) identified here as a minor sterol was recently isolated for the first time from *Brassica juncea* and its unusual side chain structure has been characterized [12].

EXPERIMENTAL

Mps were uncorr. Silica gel TLC (0.5 mm) was developed × 3 with *n*-hexane–EtOAc (6:1), and $AgNO_3$–silica gel (1:4) TLC (0.5 mm) was developed × 4 with CH_2Cl_2–CCl_4 (1:5). HPLC was carried out on a Partisil 5 ODS-2 column (Whatman, 20 cm × 8 mm i.d.; packed by Erma Optical Works, Tokyo) using a UV detector monitoring at 212 nm (mobile phase, MeOH). *RR*$_t$ on HPLC was expressed relative to cholesterol. GC on OV-17 SCOT glass capillary column was under the conditions already described [13]. MS (70 eV) were taken with a GC/MS (2% OV-17, 2 m × 3 mm i.d. glass column). ^{1}H NMR (400 MHz) spectra were determined in $CDCl_3$ with TMS as internal standard. Other techniques used in this study have been described previously [4]. Degummed *H. annuus* seed oil was courteously supplied by Dr. A. Nishioka (Ajinomoto Co., Yokohama). Origin and GC data of sterol **12** [12] and the other sterols [13] used here as the reference specimens have been described in our previous articles.

Acknowledgements—We thank Dr. Y. Fujimoto, Inst. Phys. Chem. Res. (Saitama), for ^{1}H NMR spectra. Our thanks are also due to A. Maeda for technical assistance.

REFERENCES

1. Mulheirn, L. J. (1973) *Tetrahedron Letters* 3175.
2. Nes, W. R., Krevitz, K. and Behzadan, S. (1976) *Lipids* **11**, 118.
3. Scheid, F., Rohmer, M. and Benveniste, P. (1982) *Phytochemistry* **21**, 1959.
4. Matsumoto, T., Shimizu, N., Itoh, T., Iida, T. and Nishioka, A. (1982) *J. Am. Oil Chem. Soc.* **59**, 521.
5. Matsumoto, T., Shimizu, N., Shigemoto, T., Itoh, T., Iida, T. and Nishioka, A. (1983) *Phytochemistry* **22**, 789.
6. Matsumoto, T., Shimizu, N., Asano, S. and Itoh, T. (1983) *Phytochemistry* **22**, 1830.

7. Itoh, T. and Matsumoto, T. (1978) *Yukagaku* **27**, 745.
8. Rubinstein, I., Goad, L. J., Clague, A. D. H. and Mulheirn, L. J. (1976) *Phytochemistry* **15**, 195.
9. Nes, W. R., Krevitz, K., Joseph, J., Nes, W. D., Harris, B., Gibbons, G. F. and Patterson, G. W. (1977) *Lipids* **12**, 511.
10. Chiu, P.-L. and Patterson, G. W. (1981) *Lipids* **15**, 203.
11. Matsumoto, T., Shigemoto, T. and Itoh, T. (1983) *Phytochemistry* **22**, 2622.
12. Matsumoto, T., Asano, S. and Itoh, T. (1983) *Phytochemistry* **22**, 2619.
13. Itoh, T., Tani, H., Fukushima, K., Tamura, T. and Matsumoto, T. (1982) *J. Chromatogr.* **234**, 65.

Phytochemistry, Vol. 23, No. 4, pp. 923–925, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

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

ENAMUL HOQUE

Lehrstuhl fuer Forstbotanik, Ludwig-Maximilians-Universitaet, D-8000 Muenchen 40, Amalienstr. 52, Federal Republic of Germany

(*Revised received* 29 *September* 1983)

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