Phytochemistry, 1976, Vol. 15, pp. 556–557. Pergamon Press Printed in England.

COMPARISON OF ISOPEROXIDASE PATTERNS IN TOBACCO CELL CULTURES AND IN THE INTACT PLANT*

HIDEAKI SHINSHI and MASAO NOGUCHI
Central Research Institute, The Japan Tobacco & Salt Public Corporation, 6-2 Umegaoka, Midori-ku, Yokohama, Kanagawa 227, Japan

(*Revised received* 30 *October* 1975)

Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; cell tissue culture; peroxidase.

Abstract—Analysis by ion-exchange chromatography of the enzymes from cultured tobacco cells and root or leaf tissues of the tobacco plant revealed that the cultured cells contain exclusively cationic peroxidases and the leaf tissues mainly anionic and neutral peroxidases.

INTRODUCTION

In our previous reports, the existence of multiple forms of peroxidase with different properties was demonstrated in suspension cultured tobacco cells [1,2]. In some studies, isoperoxidase patterns were shown to be tissue or organ specific [3–5]. In the present investigation, chromatographic methods were employed to clarify quantitative differences in isoperoxidase pattern between cultured cells and the corresponding intact plant.

RESULTS AND DISCUSSION

Comparison of specific activity

Analysis of the enzyme extracts prepared from leaves and roots of the tobacco plant and cultured cells, showed that cultured cells contained the higher specific activity of peroxidase, expressed either protein or fresh weight basis (Table 1).

Electrophoresis and ion-exchange chromatography

The enzyme extracts were also analyzed by gel electrophoresis (Fig. 1) and by ion-exchange chromatography on DEAE-cellulose and CM-Sephadex. Large differences were observed between the different tissues. Relative contents of anionic and neutral peroxidases and cationic peroxidases were calculated and summarized in Table 1. The cultured cells contained exclusively cationic peroxidases; on the other hand, leaf tissues predominantly contained anionic peroxidases.

Since cationic and anionic peroxidases from the cultured cells differ markedly in properties [2], the results

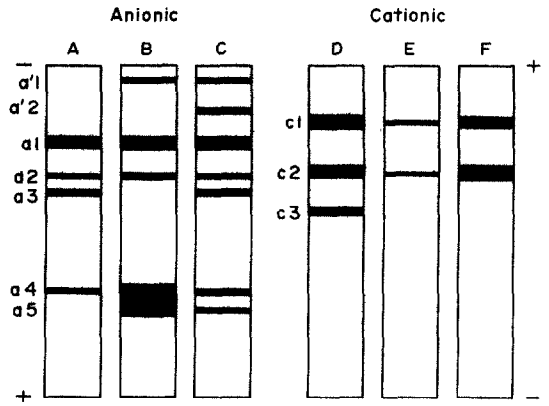


Fig. 1. Electrophoretic patterns of peroxidase in different part of tobacco. The disc electrophoresis were carried out in 7% acrylamide gels at pH 8.4 (A,B and C) and at pH 4.5 (D,E and F). The activities were stained with guaiacol. A and D, cultured cells; B and E, leaf; C and F, root.

*Part 3 in the series. Peroxidases from tobacco cell suspension culture.

Table 1. Peroxidase activities in different parts of tobacco

Plant part	Peroxidase specific activity*		Relative content†	
	units/mg protein	units/g fr. wt	Cationic peroxidases (%)	Neutral and anionic peroxidase (%)
Cultured cells	123	130	89	11
Leaf	7.1	68.4	19	81
Root	25.3	95.4	64	36

* One unit of peroxidase activity was defined as the amount which produced absorbance change at 470 nm of 1.0 per min, using guaiacol as substrate. † Relative contents were estimated from elution profile of chromatography on DEAE-cellulose and CM-Sephadex.

suggest that synthesis of anionic peroxidases is repressed and that of cationic peroxidases is induced in the cultured cells.

EXPERIMENTAL

Tobacco cells (*Nicotiana tabacum* cv. Hicks 2) were grown as described [1]. Tobacco plants were grown in a greenhouse for three months after germination. All other methods were as described previously [1].

REFERENCES

1. Shinshi, H. and Noguchi, M. (1975) *Phytochemistry* **14**, 1255.
2. Shinshi, H. and Noguchi, M. (1975) *Phytochemistry* **14**, 2141.
3. Macnicol, P. K. (1966) *Arch. Biochem. Biophys.* **117**, 347.
4. Siegel, B. Z. and Galston, A. W. (1967) *Plant Physiol.* **42**, 221.
5. Sheen, S. J. (1969) *Phytochemistry* **8**, 1839.

Phytochemistry, 1976, Vol. 15, pp. 557–558. Pergamon Press. Printed in England

THE CONSTITUTION OF ISOCENTDAROL, A SESQUITERPENEDIOL FROM *CEDRUS DEODARA**

D. K. KULSHRESHTHA and R. P. RASTOGI
Central Drug Research Institute, Lucknow, India

(*Received* 30 *August* 1975)

Key Word Index—*Cedrus deodara*; Pinaceae centdarol; isocentdarol; sesquiterpene diol; spasmolytic agent.

The isolation of the spasmolytic constituents [1, 2] from the hexane-soluble extractive of the wood of *Cedrus deodara* and the structure elucidation of centdarol [3] was reported in earlier communications. The present paper deals with chemical studies on isocentdarol.

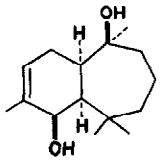
Isocentdarol. $C_{15}H_{26}O_2$ (M^+ 238), showed IR bands at 3344, 1037 (OH), 817 cm^{-1} (C=CH) and, like centdarol, exhibited PMR signals for three tert. C–Me, a vinylic Me, a carbinol H, two OH and a vinylic H. Acetylation yielded a monoacetate, $C_{17}H_{28}O_3$ ($M-H_2O$ *m/e* 262), whose PMR spectrum showed an acetoxyl singlet at 2.07 ppm and a downfield shift by 1.22 ppm of the carbinol H indicating the presence of a secondary OH group in the molecule.

In the PMR spectrum of isocentdarol on addition of TAI [4], the 2H signal due to OH groups at 2.08 ppm was replaced by a 2H multiplet of carbamate protons at 8.7 ppm with concurrent shifts of the carbinol signal from 4.06 to 5.38 ppm, vinyl H from 5.78 to 5.98 ppm and a tertiary C–Me from 1.26 to 1.66 ppm. Thus the presence of two OH groups in the molecule was confirmed. The PMR spectrum of isocentdarol acetate in presence of TAI, however, showed only 1H carbamate signal at 8.61 ppm without any shift of the vinylic H multiplet but a paramagnetic shift of the tert. C–Me singlet by 0.38 ppm. This demonstrated the position of the secondary OH group as allylic and that of the tertiary OH group on the carbon bearing the tertiary C–Me.

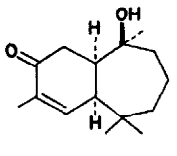
Isocentdarol on chromic acid oxidation, furnished a monoketo derivative, (isocentdarone) $C_{15}H_{24}O_2$ (M^+ 236), which showed enone IR absorption (1670 cm^{-1}) and λ_{max} (245 nm, ε 6162) consistent with the allylic nature of the secondary OH group in the molecule.

Catalytic hydrogenation of isocentdarol yielded a dihydroproduct, $C_{15}H_{28}O_2$ (M^+-18 *m/e* 222), which did not show vinylic Me and vinylic H signals in the PMR spectrum indicating that both were located on the same double bond in isocentdarol.

From the above data it was evident that isocentdarol was an isomer of centdarol (1) differing in the position of the double bond and the secondary OH group. The double bond could be placed at Δ^2 in view of the similar splitting pattern (*dm*. *J* 5 Hz) of the vinylic H signal in the PMR spectra of both isocentdarol and himachalol [5]. The secondary OH group would, therefore, be located at C_4. This was in agreement with large downfield shift (1.18 ppm) of the vinylic H in the PMR spectrum of isocentdarone, which also exhibited a 2H doublet (*J* 3 Hz) at 2.50 ppm and a 1H multiplet at 2.75 ppm assignable to C_5-methylene and C_1-methine protons respectively. The solvent induced shifts in the PMR spectra of isocentdarone (2) in benzene d_6 and pyridine d_5 were also in accord with the proposed assignments.



(1)



(2)

In order to confirm the structure, isocentdarol acetate was hydroxylated with OsO_4 to give a triol acetate (3), $C_{17}H_{30}O_5$ which did not show any vinylic Me and H signals in the PMR spectrum but an additional Me on an oxygen-bearing carbon at 1.35 ppm, a carbinol H doublet (*J* 8 Hz) at 3.67 ppm and a methine H (under an acetoxyl group) as a quartet (*J* 11 and 5 Hz) at 4.93 ppm. On reaction with periodate the triol acetate (3) furnished two major *seco*-products, A and B, which were separated by chromatography on silica gel.

* Part 4 in series, Chemical Examination of *Cedrus deodara* Loud. See ref. [3] for Part 3. CDRI Communication No.