Ethyl 2,4-Bis(2,4-dichlorophenoxy)acetoacetate——To a solution of 2 g. of 3,5-dimethylpyrazole and 0.4 g. of sodium in 20 ml. of tetrahydrofuran was added 5.6 g. of ethyl (2,4-dichlorophenoxy)acetate. After being stirred for 30 min. at room temperature, 300 ml. of H_2O was added to the mixture and the separated solid was collected (sodio-compound, m.p. 217°) and then suspended in warm acetone. Acidification with 10% aq. HCl and recrystallization from *n*-hexane gave colorless crystals, m.p. 97～98°; yield, 3.8 g.

(2-Hydroxymethyl-4-chlorophenoxy)acetic Acid Hydrazide——A mixture of 4.9 g. of ethyl 2-hydroxymethyl-4-dichlorophenoxy)acetate and 1.5 g. of hydrazine hydrate (80%) was refluxed in 20 ml. of EtOH for 2 hr. After cooling, the separated solid was collected and recrystallized from DMF-H_2O to give colorless needles, m.p. 151.5°; yield, 4 g. (87%). *Anal.* Calcd. for $C_9H_{11}O_3N_2Cl$: C, 46.86; H, 4.81; N, 12.15. Found: C, 46.87; H, 4.79; N, 11.95.

The authors wish to express their deep gratitude to Dr. S. Tatsuoka, Director of this division, and to Takeda Chemical Industries, Ltd. for permission to publish this work. Grateful acknowledgment is made to Dr. T. Harukawa of this division for his encouragement.

Thanks are also due to Mr. M. Kan for elemental analyses, to Miss F. Kasahara for NMR measurement, and to Mr. K. Maki for his assistance in the experimental work.

Summary

In view of recently reported chemical reactivities of the azolides, a number of 1-aryloxyacylpyrazoles were synthesized for their evaluation as plant growth regulator.

The structure of 1-aryloxyacylpyrazoles derived from unsymmetrical pyrazoles was discussed on the basis of nuclear magnetic resonance data.

Some reactions of 1-aryloxyacylpyrazoles were also described.

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91. Kōtaro Takahashi and Toshie Nakagawa: Studies on Constituents of Medicinal Plants. Ⅷ.*1 The Stereochemistry of Paulownin and Isopaulownin.

(*Faculty of Pharmaceutical Sciences, Kanazawa University**2)

Paulownin,[1] $C_{20}H_{18}O_7$, m.p. 105～106°, $[\alpha]_D$ =29.0, a new lignan, isolated together with *d*-sesamin, from the wood of *Paulownia tomentosa* (kiri) has been established as 1,4-bis(3,4-methylenedioxyphenyl)-tetrahydro-1*H*,3*H*-furo[3,4-*c*]furan-3*a*-ol. The present paper deals with certain nuclear magnetic resonance (NMR) evidence for the assignment of the stereochemistry of *d*-sesamin (Ia) and *d*-asarinin (Ib), and with the stereochemistry of paulownin (Ⅱa) and isopaulownin (Ⅱb), m.p. 132°, $[\alpha]_D$ =127.0°, the latter of which was derived from paulownin. Paulownin was refluxed with 20% formic acid or 5% ethanol-hydrochloric acid to give isopaulownin, $C_{20}H_{18}O_7$, which gave a monoacetate $C_{22}H_{20}O_8$, m.p. 105°, $[\alpha]_D$ =83.1°. The ultraviolet spectrum of Ⅱb is superimposable with that of Ⅱa.

The NMR spectra of *d*-sesamin and *d*-asarinin has been reported by Jones, *et al.*[2]

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*1 A part of this study was presented at the XXVth International Congress of the Pharmaceutical Sciences (F. I. P.), Prague, August 25, 1965. Part Ⅶ: Yakugaku Zasshi, **86**, 441 (1966).

*2 Takara-machi, Kanazawa (高橋幸太郎, 中川俊江).

1) K. Takahashi, T. Nakagawa: Yakugaku Zasshi, **83**, 1101 (1963).

2) W. A. Jones, M. Beroza, B. D. Becker: J. Org. Chem., **27**, 3232 (1963).

and by us[1] and recently Birch, *et al.*[3] presented the stereochemistry of *d*-sesamin and *d*-asarinin as Ia and Ib, respectively, from the study on their optical rotation, however, whether *d*-sesamin should be formulated as Ia has not been determined conclusively. So it seems to be necessary for us to begin our study with reexamination of their spectra in connection with the NMR spectral analysis of paulownin and isopaulownin. The NMR spectra of Ia, Ib, IIa, and IIb*[3] are shown in Fig. 1.*[3]

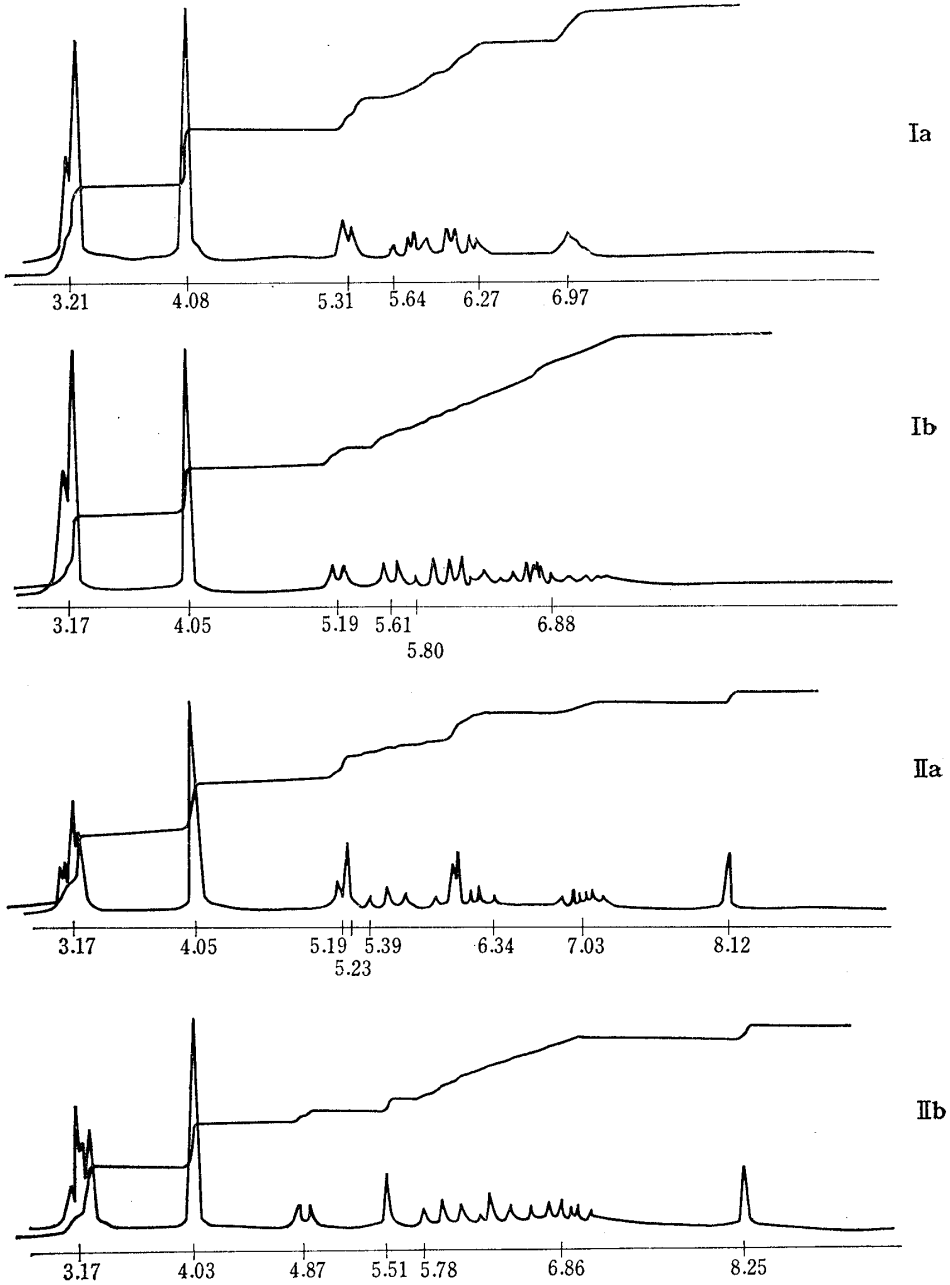
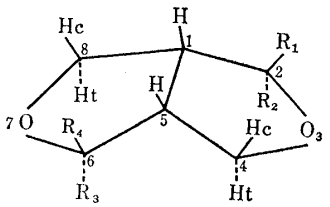


Fig. 1. The NMR spectra of *d*-sesamin (Ia), *d*-asarinin (Ib), paulownin (IIa) and isopaulownin (IIb).

*[3] The NMR spectra were measured by the Varian Associates NMR spectrometer at 60 Mc. in $CDCl_3$, using tetramethylsilane as internal reference. The position of resonances were given as τ-values.

3) A. J. Birch, M. Smith : J. Chem. Soc., **1964**, 2705, 2709.

d-Sesamin and d-Asarinin



Ia : $R_1=R_4$=3,4-methylenedioxyphenyl
$R_2=H_t$, $R_3=H_t$
Ib : $R_1=R_3$=3,4-methylenedioxyphenyl
$R_2=H_t$, $R_4=H_c$
Ic : $R_2=R_3$=3,4-methylenedioxyphenyl
$R_1=H_c$, $R_4=H_c$

The NMR spectrum of *d*-sesamin (Fig. 2) shows a doublet at 5.31 p.p.m. (J=4.8 c.p.s., 2 protons), which would be assigned to equivalent protons C_2-H_t and C_6-H_t of Ia, of which the former proton is split by coupling with C_1-H and the latter, by coupling with C_5-H, respectively. The coupling constant J=4.8 c.p.s. of the doublet indicates that C_2-H_t and C_1-H and also C_6-H_t and C_5-H are in a *trans* configuration, respectively.

The NMR spectrum of *d*-asarinin (Fig. 3) shows two doublets at 5.19 p.p.m. (J=4.8 c.p.s., one proton) and at 5.61 p.p.m. (J=7.2 c.p.s., one proton), which would be assigned to non-equivalent protons, C_2-H_t and C_6-H_c of Ib, of which the former is split by coupling with C_1-H and the latter, by coupling with C_5-H. The coupling constant J=4.8 c.p.s. of the doublet of the lower field (5.19 p.p.m.) suggests that C_2-H_t and C_1-H remain in a *trans* configuration and the coupling constant J=7.2 c.p.s. of the doublet of the higher field (5.61 p.p.m.) suggests that C_6-H_c and C_5-H are in a *cis* configuration.

These data indicate that the configurational change of 3,4-methylenedioxyphenyl and the proton at C_6 results in the upfield shift of the signal by the changed proton at C_6 and the downfield shift of the signal by the unchanged proton at C_2, which might be due to the anisotropy of the phenyl ring.

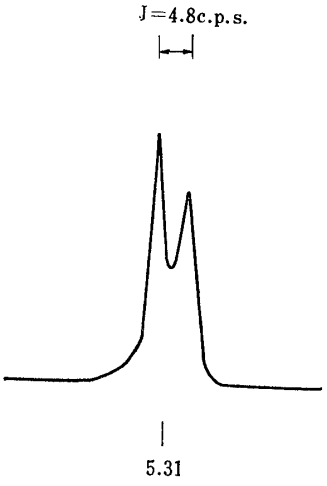


Fig. 2.

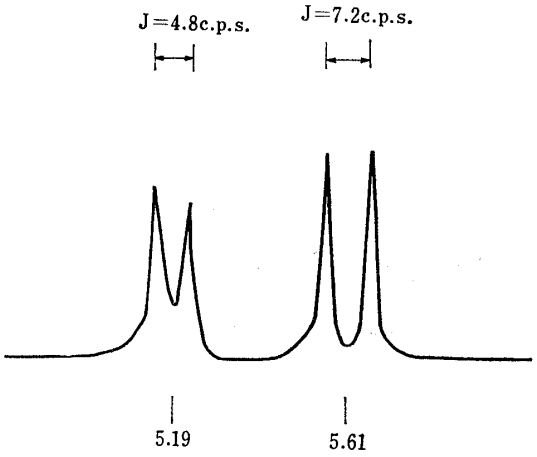


Fig. 3.

The NMR spectrum of *d*-sesamin (Fig. 4) also exhibits two quartets at 5.64~5.91 p.p.m. (center 5.78 p.p.m., 2 protons) and at 6.07~6.27 p.p.m. (center 6.17 p.p.m., 2 protons), which would be assigned to two pairs of equivalent protons C_4-H_c and C_8-H_c, and C_4-H_t and C_8-H_t of Ia, respectively. A possible explanation is as follows (Fig. 4) :

C_4-H_t and C_8-H_t, and also C_4-H_c and C_8-H_c are equivalent, respectively, however, C-H_t and C-H_c are non-equivalent and have different chemical shifts. C_4-H_c is coupled with C_4-H_t to give a doublet (J=9.2 c.p.s.) and each peak of the doublet in the lower field (due to C_4-H_c) is split by coupling with C_5-H to give doublets (J=7.0 c.p.s.), finally C_4-H_c exhibits a quartet at 5.64~5.91 p.p.m. The observed coupling constant J=7.0 c.p.s. suggests that C_4-H_c and C_5-H are in a *cis* configuration. C_8-H_c, which is equivalent

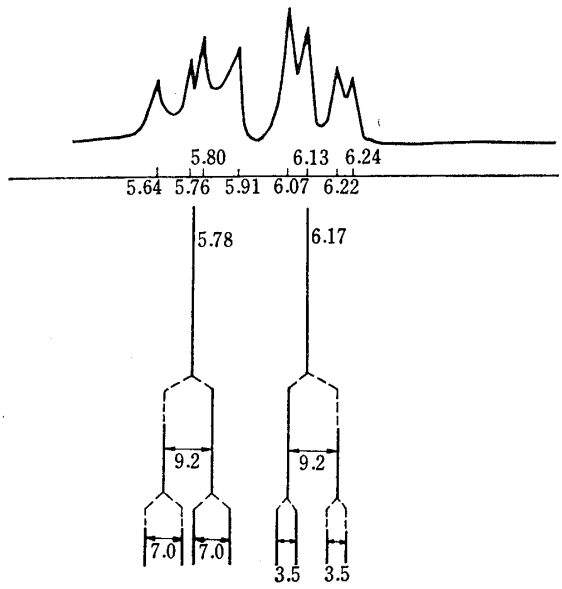


Fig. 4.

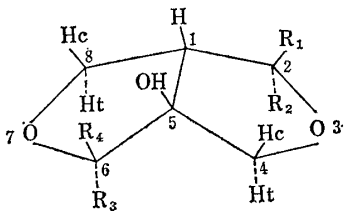
to C_4-H_c also exhibits a similar quartet. On the other hand, C_4-H_t is coupled with C_4-H_c to give a doublet (J=9.2 c.p.s.) and then each peak of the doublet in the higher field (due to C_4-H_t) is again split by coupling with C_5-H to give doublets (J=3.5 c.p.s.), finally C_4-H_t shows a quartet at 6.07~6.27 p.p.m. The observed coupling constant J=3.5 c.p.s. suggests that C_4-H_t and C_5-H are in a *trans* configuration. C_8-H_t, which is equivalent to C_4-H_t also exhibits a similar quartet. These data are almost in agreement with those of pinoresinol, reported by Ludwig[4] as follows :

H_c=5.76 p.p.m., H_t=6.17 p.p.m., $J(H_c-H_t)$=9.23 c.p.s.,
$J(C_4\text{-}H_c\cdots C_5\text{-}H)=J(C_8\text{-}H_c\cdots C_1\text{-}H)$=7.0 c.p.s.,
$J(C_4\text{-}H_t\cdots C_5\text{-}H)=J(C_8\text{-}H_t\cdots C_1\text{-}H)$=3.8 c.p.s.

The methylene protons at C_4 and C_8 of *d*-asarinin shows a complex multiplet at 5.80~6.88 p.p.m., which is rather in high field than that of *d*-sesamin. The above mentioned NMR data support the configurational assignment of *d*-sesamin and *d*-asarinin as Ia and Ib, respectively, which is in agreement with the result by Birch.[3]

Paulownin and Isopaulownin

The assignment of the stereochemistry of paulownin and isopaulownin as IIa and IIb can be made by their NMR analysis as follows :



IIa : R_1=R_4=3,4-methylenedioxyphenyl,
R_2=H_t, R_3=H_t

IIb : R_1=R_3=3,4-methylenedioxyphenyl,
R_2=H_t, R_4=H_c

The spectrum of paulownin (Fig. 5) exhibits two signals at 5.15 p.p.m. and 5.23 p.p.m., which are assigned to the protons at C_2 and C_6 and these two signals are equivalent to two protons, it is considered that the signal at 5.15 p.p.m. comes from a doublet with a hidden signal in the signal at 5.23 p.p.m. The fact that the J of this doublet is about 4.8 c.p.s. and not larger than 5.5 c.p.s. suggests that the proton at C_2 and the proton at C_1 are in a *trans* configuration, and the signal at 5.23 p.p.m. is assigned to the proton at C_6, as shown in IIa.

The spectrum of isopaulownin (Fig. 6) shows a doublet at 4.87 p.p.m. (one proton), which could be assigned to C_2-H_t and a singlet at 5.51 p.p.m. (one proton), which could be assigned to C_6-H_c. As is observed in the case of *d*-sesamin and *d*-asarinin, C_2-H_t signal of isoform shifts to the lower field and C_6-H_c of isoform to the higher

4) H. Ludwig, B. J. Nist, J. L. McCarthy : J. Am. Chem. Soc., **86**, 1186 (1964).

field. The observed coupling constant J=5.0 c.p.s. of C_2-H_t suggests that the stereochemistry of C_2-H_t and C_1-H is unchanged in a *trans* configuration, however, that of C_6-H_c and C_5-OH is in a *cis* configuration as shown in IIb.

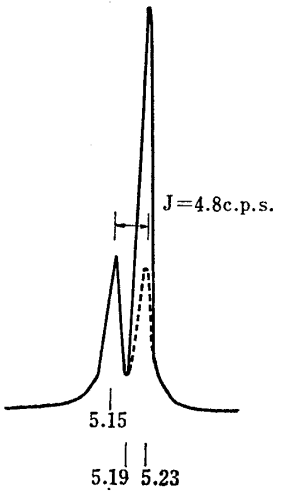


Fig. 5.

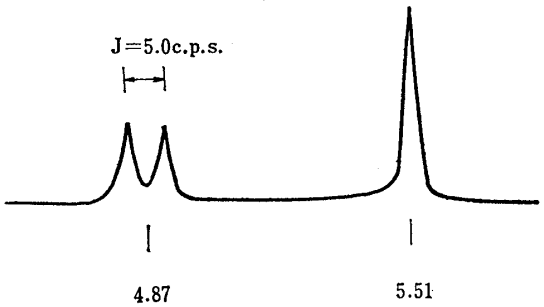


Fig. 6.

The spectrum of paulownin exhibits a complicated, but symmetrical sextet at 6.87 p.p.m., 6.95 p.p.m., 7.00 p.p.m., 7.04 p.p.m., 7.08 p.p.m., and 7.18 p.p.m. (center 7.03 p.p.m., one proton) (Fig. 7), which would be assigned to the proton at C_1. This would be analyzed as follows :

The proton at C_1 is first split by coupling with C_8-H_c to give a doublet (J=8.0 c.p.s.) and then each of peaks is split by coupling with C_2-H_t to give a quartet (J=5.0 c.p.s.) and finally by coupling with C_8-H_t to give an octet (J=5.5 c.p.s.). The signal appears as a sextet, which actually is shown in Fig. 7.

The methylene protons of paulownin give a complicated and asymmetrical multiplet between 5.39 p.p.m. and 6.34 p.p.m. (4 protons), of which the sum of intensities of signals at 5.39 p.p.m., 5.53 p.p.m., and 5.67 p.p.m. are equivalent to one proton and the sum of intensities of signals at 5.89 p.p.m., 6.05 p.p.m., 6.09 p.p.m., 6.18 p.p.m., 6.24 p.p.m., and 6.34 p.p.m. are equivalent to three protons. This multiplet might be tentatively analyzed as follows (Fig. 8).

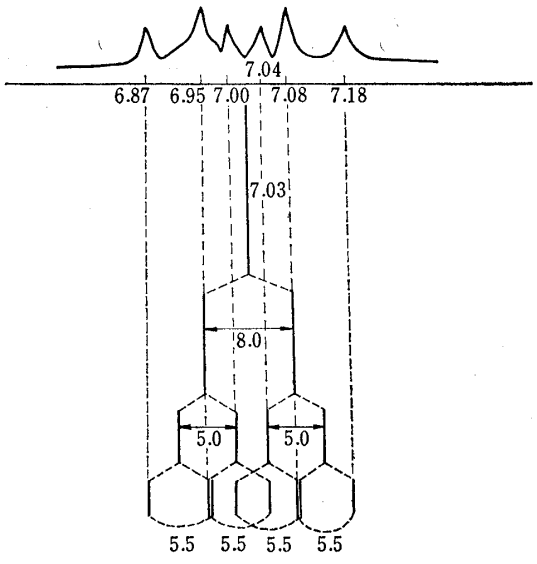


Fig. 7.

The methylene protons at C_8, C_8-H_c and C_8-H_t are non-equivalent, as are those of *d*-sesamin, and have different chemical shifts, showing characteristic signals at 5.53 p.p.m. and 6.21 p.p.m., respectively. These protons are coupled with each other to give two doublets (J=9.0 c.p.s.). The lower field doublet (due to C_8-H_c) is further coupled with C_1-H to give a quartet (J=8.0 c.p.s.) and higher field doublet (due to C_8-H_t) is also coupled with C_1-H to give a quartet (J=5.5 c.p.s.). C_4-H_c and C_4-H_t, which are also non-equivalent and have different chemical shifts, are coupled with each other to give a quartet (J=9.3 c.p.s.) between 5.89 p.p.m. and 6.24 p.p.m. These signals are synthesized to give a multiplet which is almost equal to that of paulownin. The assumption that

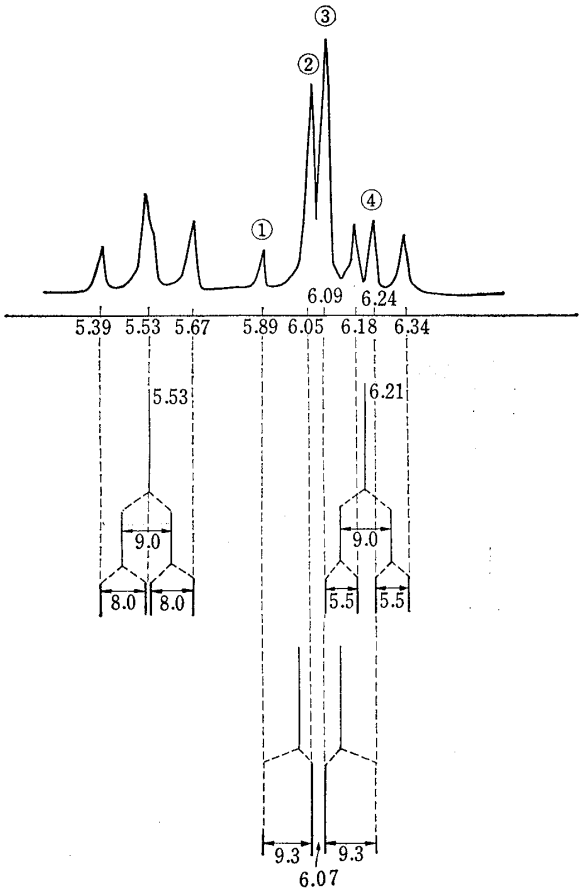


Fig. 8.

the methylene protons at C_4,C_4-H_t and C_4-H_c are of AB type and show signals at 5.89 p.p.m. (signal 1), 6.05 p.p.m. (signal 2), 6.09 p.p.m. (signal 3), and 6.24 p.p.m.(signal 4) (O point is at 6.07 p.p.m.), might be supported by the following calculation.

$$4-3=2-1=J_{AB} \qquad (1)$$

The observed value of (2-1) is 0.16 p.p.m. and that of (4-3) is 0.15 p.p.m.

$$3-1=4-2=\sqrt{(\delta_B-\delta_A)^2-J^2_{AB}} \qquad (2)$$

The observed value of (3-1) is 0.20 p.p.m. and (4-2) is 0.19 p.p.m. Using the value of J_{AB} as 0.16 p.p.m. and that of (3-1) as 0.20 p.p.m., the following equation is derived from the equation 2,

$$0.04=(\delta_B-\delta_A)^2-0.16^2$$

and so $\delta_B-\delta_A=0.12$ p.p.m.

Consequently,

$$\delta_A=6.07-\frac{1}{2}\times 0.12=6.01 \text{ p.p.m.}$$

$$\delta_B=6.07+\frac{1}{2}\times 0.12=6.13 \text{ p.p.m.}$$

The relative intensities are given by (3) and (4), where J_{AB} is 0.16 p.p.m. and $(\delta_B-\delta_A)$ is 0.12 p.p.m.,

$$1=4=1-J_{AB}[(\delta_B-\delta_A)^2-J^2_{AB}]^{-1/2}=0.2 \qquad (3)$$

$$2=3=1+J_{AB}[(\delta_B-\delta_A)^2-J^2_{AB}]^{-1/2}=1.8 \qquad (4)$$

Thus, the relative intensities of both signals 1 and 4 are 0.2 and those of both signals 2 and 3 are 1.8, indicating that the ratio of the relative intensity of signal 1 to that of the signal 2 is 1/9. This value is well in accord with the observed value about 1/9 of the spectrum.

Difference in chemical shifts of C_4-methylene and C_8-methylene protons may be derived from a sum of effects, shielding and deshielding of two phenyl rings and one hydroxyl group in the compound. The exact factor which may govern the NMR information of these protons is under way.

The methylene signals of isopaulownin is observed in a rather high field at 5.78 ~6.86 p.p.m. (4 protons) than that of paulownin, which might be due to the anisotropy of the phenyl ring. Its analysis is not completed yet. The signals of the phenyl protons, methylenedioxy protons and hydroxy proton are observed[1] as shown in Fig. 1. All these NMR data support the configurational assignment of paulownin and isopaulownin as IIa and IIb, respectively. In this case, the configurational change is considered to have taken at C_6 which is neighbored to C_5-OH. This conclusion is supported by the observed change in the optical rotation,[3] that is, the observed values of optical rotation for paulownin and isopaulownin are +29.0°, and +127.0°, respectively. This change with difference +98.0° corresponds with the change in the optical rotation between *d*-sesamin and *d*-asarinin with the difference of +49.0°.

Consequently, the stereochemistry of *d*-sesamin, *d*-asarinin, paulownin and isopaulownin could be elucidated as Ia, Ib, IIa and IIb, respectively, and epi-asarinin as Ic.

Experimental

Isomerization of Paulownin to Isopaulownin——a) Paulownin (2.5 g.), dissolved in 120 g. of 50% EtOH-HCl (w/w) solution, was refluxed for 7 hr. After cooling, the solvent was distilled off to give brown substance, which was, after recrystallization from CH_3OH, separated into paulownin and isopaulownin by the thin-layer chromatography (the dry method) as follows. The substance (50 mg.), after drying completely, dissolved in a minimum volume of $CHCl_3$, was spotted on a glass plate (20×5 cm.), which was covered with alumina (thickness 0.5 mm.) and the plate was twice developed with ethylacetate-benzene solution (1:3, v/v). After drying, the plate showed two bands under UV illumination, of which the lower band was removed off and extracted with $CHCl_3$ and $CHCl_3$ solution was evaporated to give white crystals, which was mainly consisted of isopaulownin. Thus obtained isopanlownin was again chromatographed as mentioned above to give pure isopaulownin as white rhombic crystals, $C_{20}H_{18}O_7$, m.p. 132°, $[\alpha]_D=127.0°$ (c=0.795, $CHCl_3$), from methanol. Yield, 15%. Isopaulownin gave a superimposable UV[*4] curve with that of paulownin. UV λ_{max}^{EtOH} mμ (ε): 237.5 (9180), 287 (8160). IR[*4] ν_{max} cm^{-1} : 3400, 2860, 1610, 1510, 1500, 1450, 1400, 1375, 1360, 1300, 1270, 1250, 1210, 1190, 1105, 1060, 1040, 1030, 1000, 970, 940, 886, 830, 815, 795, 785, 746, 720. *Anal.* Calcd. for $C_{20}H_{18}O_7$: C, 64.86 ; H, 4.90. Found : C, 64.98 ; H, 4.72.

b) Paulownin (0.3 g.), suspended in 50 ml. of 20% formic acid, was refluxed for 10 hr. and then formic acid solution was evaporated *in vacuo* to give brown substance, which was purified by the thin-layer chromatography as mentioned above, to give isopaulownin, m.p. 132°. This was proved to be identical with isopaulownin, mentioned above, by the mixed melting determination, UV and IR spectra.

Acetylation of Isopaulownin——A mixture of 0.2 g. of isopaulownin, 3 ml. of Ac_2O and 0.2 g. of CH_3COONa was gently refluxed on an oil bath for 2 hr. and after cooling, the mixture was poured into ice water. After standing overnight in an ice-box, the precipitates were filtered, dried and then recrystallized from methanol to give white crystalline substances, m.p. 105°, $[\alpha]_D=83.1°$ (c=0.830, $CHCl_3$). UV λ_{max}^{EtOH} mμ (ε) : 238.5 (9120), 288 (8020). IR ν_{max} cm^{-1} : 2880, 1730, 1620 (w, broad), 1510, 1500, 1450, 1380, 1350, 1250, 1240, 1190, 1110, 1065, 1040, 945, 870, 830, 815, 795, 745, 720. *Anal.* Calcd. for $C_{22}H_{20}O_8$: C, 64.07; H, 4.89. Found : C, 64.10; H, 4.86.

Isomerisation of *d*-Sesamin to *d*-Asarinin——According to the method by Beroza,[5] 1 g. of *d*-sesamin was refluxed for 16 hr. with 50 g. of EtOH-HCl solution (10% w/w) to give *d*-asarinin, which was purified by the thin-layer chromatography as in the case of isopaulownin, m.p. 121.5°, $[\alpha]_D=121°$ (c=0.9, $CHCl_3$). UV λ_{max}^{EtOH} mμ (ε) : 237.5 (9010), 288 (8180). *Anal.* Calcd. for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found : C, 67.45; H, 5.31.

The authors wish to thank Dr. S. Matsuoka of this university and the Research Laboratory of Takeda Pharmaceutical Industries, Ltd. for the measurement of NMR spectra. Thanks are due to Mr. Y. Itatani for elemental analysis and also due to the Ministry of Education for a Grant-in-Aid for Scientific Research.

Summary

The stereochemistry of paulownin, isopaulownin, m.p. 132°, which was derived from paulownin with 20% EtOH-HCl or 5% HCOOH, *d*-sesamin and *d*-asarinin were elucidated as IIa, IIb, Ia and Ib by the analysis of their NMR spectra, respectively and consequently, epi-asarinin as Ic.

(Received October 28, 1965)

[*4] Ultraviolet absorption spectra were taken in ethanol solution using Hitachi EPU-2A spectrophotometer. Infrared absorption spectra were taken in KBr pellet, if not otherwise stated. by Nippon Bunko Model IRS infra code.

5) M. Beroza : J. Am. Chem. Soc., 78, 5082 (1956).