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Stereochemical Studies of the 4-Hydroxyl Group of Polymethoxyflavan-3,4-diols

Shinzaburo Hishida, Misao Umehara, Fujiyuki Kayanuma
and Ryoichi Miyazaki

Department of Chemistry, Nihon University, Setagaya-ku, Tokyo

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In the previous papers, it was suggested that the 4-hydroxyl group of flavan-3,4-*trans*-diols altered in configuration upon the formation of the isopropylidene derivatives. In order to confirm this suggestion, the isopropylidene derivatives were prepared by the reaction of 4-^{16}OH with acetone-^{18}O, and of 4-^{18}OH with acetone-^{16}O, and the contents of oxygen-18 were analyzed. The oxygen atom from acetone was found in the isopropylidene derivatives of *trans*-3′,4′,5,7-tetramethoxy-*cis*- and *trans*-3′,4′,5′,5,7-pentamethoxy-flavan-3,4-diols.

The formation of isopropylidene derivatives has frequently been used for the assignment of vicinal dihydroxyl groups in a cyclohexane ring to a cis configuration. However, it has been shown that this procedure is not diagnostic for a cis configuration in the case of 3,4-dihydroxyl groups of flavan-3,4-diols.[1] We have shown that identical isopropylidene derivatives were obtained from pairs of flavan-3,4-*cis*- and *trans*-diols which had methoxyl groups at the 5,7-position. Non-substituted flavan-3,4-*cis*-diol, its 4′-methoxy, and 7-methoxy derivatives gave the respective isopropylidene derivatives, but their trans isomers gave no isopropylidene derivatives by the reaction of flavan-3,4-diols with acetone in the presence of cupric sulfate.[2] In contrast to them, the trans isomers of 3′,4′,5,7-tetramethoxy- and 3′,4′,5′,5,7-pentamethoxyflavan-3,4-diol afforded the same isopropylidene derivatives as those from the respective cis isomers.[3] This anomaly may be explained by the benzylic character at the 4-position, which was enhanced by the presence of methoxyl groups at the 5,7-position and which then facilitated the formation of carbonium ions and the attack of the oxygen atom of acetone from the less strained site.[4]

We will report here the determination of the origin of the oxygen atom at the 4-position of the isopropylidene derivatives, whether it is from the 4-hydroxyl group or from acetone. The isopropylidene derivatives were prepared from 3′,4′,5,7-tetramethoxyflavan-3,4-*cis*- and *trans*-diol, which contained natural oxygen atoms, as a result of a reaction with acetone enriched with oxygen-18 in the presence of cupric sulfate, and from 3′,4′,5′,5,7-pentamethoxyflavan-3,4-*cis*- and *trans*-diol, which contained natural oxygen atoms, as a result of a reaction with enriched acetone. 3′,4′,5′,5,7-Pentamethoxyflavan-3,4-*cis*- and *trans*-diol, which were enriched at the 4-hydroxyl group with oxygen-18, were prepared by the reduction of the corresponding 3-hydroxyflavanone (ampelopsin pentamethyl ether), enriched with oxygen-18 at the 4-carbonyl oxygen atom, and submitted to a reaction with natural acetone.

Results and Discussion

The results of the measurements of the oxygen-18 contents are summarized in Table 1. The values of the oxygen-18 contents were calculated from the $M+2/M$ ratio of the isopropylidene derivatives[5] and carbon dioxides,[6] which were obtained by the combustion of the samples, in the mass-spectra. It seems that the values from carbon dioxides are more appropriate, because in them the undesirable disturbance of contaminated deuterium atoms is excluded.

The cis and trans isomers of 3′,4′,5,7-tetramethoxyflavan-3,4-diols were readily separated through the formation of the boron complex of the cis isomer.

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[2] S. Fujise, T. Munekata, E. Ishikawa, T. Kobayashi, I. Sakai, M. Ueno, T. Yuki and S. Hishida, *Nippon Kagaku Zasshi* (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **84**, 81 (1963).

[3] S. Fujise, K. Adachi and S. Hishida, *ibid.*, **83**, 1294 (1962); S. Fujise, T. Onuma and S. Hishida, *ibid.*, **83**, 1298 (1962).

[4] S. Fujise, Y. Fujise and S. Hishida, *ibid.*, **84**, 78 (1963).

[5] G. A. Hamilton and F. H. Westheimer, *J. Am. Chem. Soc.*, **81**, 6332 (1959); G. Swain, G. Tsuchihashi and L. J. Taylor, *Anal. Chem.*, **35**, 1415 (1963).

[6] S. Oae, T. Kitao and Y. Kitaoka, *J. Am. Chem. Soc.*, **84**, 3359 (1962) (*cf.* D. Rittenberg and L. Ponticorvo, *J. Appl. Rad. Isotopes*, **1**, 208 (1956)).

TABLE 1. THE CONTENTS OF OXYGEN-18 AT THE 4-POSITION

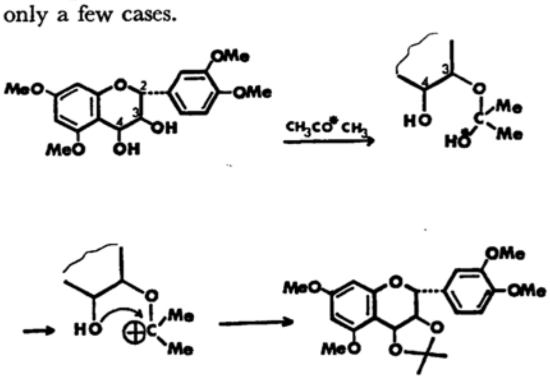
	Methoxyflavan-3,4-diols	Acetone	Isopropylidene derivatives
3′,4′,5,7-$(OMe)_4$-			
cis-diol	0.2(nat.)	1.45	0.2(0.2)
trans-diol	0.2(nat.)	1.45	1.4(1.7)
3′,4′,5′,5,7-$(OMe)_5$-			
cis-diol	0.2(nat.)	2.7	2.7(3.2)
	4.6(6.1)	0.2(nat.)	0.2(0.2)
trans-diol	0.2(nat.)	2.7	2.7(2.6)
	4.8(5.1)	0.2(nat.)	0.2(0.2)

The values were calculated from the M+2/M ratio of CO_2. The values in parenthesis were calculated from the M+2/M ratio of flavan-3,4-diols and isopropylidene derivatives.

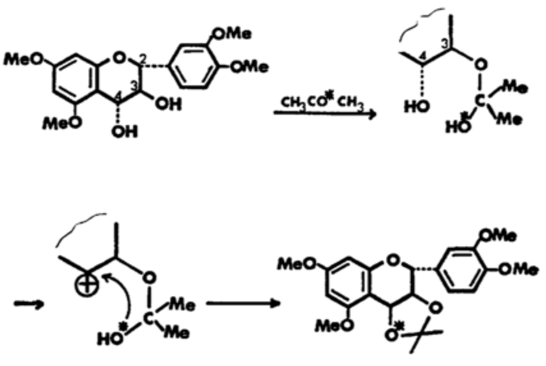
After the reduction of 3′,4′,5,7-tetramethoxy-3-hydroxyflavanone with sodium borohydride in boiling methanol, the reaction mixture was concentrated to a small volume and diluted with water; then the crystallization of the pure trans isomer took place. The aqueous solution which contained the boron complex of cis isomer gave the pure cis isomer on acidification with acetic acid. Their purities were checked by thin-layer chromatography. The distinct separation of the spots of the cis and trans isomers on a chromatogram was achieved by using silica gel treated with sodium borate. An incomplete separation of the cis and trans isomers was observed when 3′,4′,5′,5,7-pentamethoxy-3-hydroxyflavanone was reduced with sodium borohydride. According to the same procedure, the cis isomer obtained on the aqueous solution was found to be pure by thin layer chromatography, but the trans isomer proved to be contaminated with the cis isomer. The purification of the trans isomer was difficult, and the impure sample was submitted to the oxygen-18 experiment.

From Table 1, it is evident that the isopropylidene derivative from the cis isomer of tetramethoxyflavan-3,4-diol contained no oxygen-18 atom of acetone; it is supposed to be formed with a retention of the 4-hydroxyl group through the normal process (A).

The S_N1-type mechanism was postulated, because all the experimental results were covered by using the formation of carbonium ions. However, the S_N2-type and S_Ni-type mechanisms were valid in only a few cases.



However, it was found that the trans isomer readily formed the identical isopropylidene derivative incorporated with the oxygen-18 of acetone. This anomalous reaction may be explained as follows (process (B));



The formation of the dioxolane ring from the flavan-3,4-*trans*-diol through the process (A) requires more distortion of the pyran ring than that from the flavan-3,4-*cis*-diol. The dioxolane ring should be formed through the process that requires the less distortion. The enhanced benzylic character at the 4-carbon atom by the methoxyl groups facilitates the formation of carbonium ions, and the hydroxyl group of acetone hemiketal more readily attacks the carbonium ion from the less distorted site, the cis-direction, than from the trans-direction.

Both cis and trans isomers of pentamethoxyflavan-3,4-diol gave the same isopropylidene derivative, which was incorporated with acetone oxygen. Although the trans isomer was impure because of contamination with the cis isomer, the incorporation of acetone oxygen is not unexpected (*via* process (B)). However, the fact that the cis isomer incorporated acetone oxygen is very interesting and shows that the formation of carbonium ions at the 4-carbon atom and the reaction of the 4-carbonium ion with the hydroxyl group of acetone hemiketal (process (B)) exceeds the normal process (A).

The readiness of the formation of the 4-carbonium ion and of the attack of the hydroxyl group of acetone hemiketal in the case of pentamethoxyflavan-3,4-diol, relative to the case of the tetramethoxy derivative, is not clearly explained by the resonance stabilization and the steric factors of the reaction process.

Experimental

3′,4′,5,7-Tetramethoxyflavan-3,4-diols. As has been described in the previous papers,[3] 3′,4′,5,7-tetramethoxy-3-hydroxyflavanone was reduced with sodium borohydride (in a large excess) in boiling methanol. The reaction mixture was concentrated to a small volume *in vacuo* and diluted with water. The pure trans isomer soon began to crystallize, and it separated from methanol as prisms, mp 205—206°C. On the acidification of the aqueous solution with acetic acid, the cis isomer was separated in a gelatinous form; it was changed to needles when shaken with ether, and was separated from methanol as needles, mp 165—166°C.

Thin-layer chromatography on silica-gel, using ethyl acetate saturated with water, showed that the cis and trans isomers were both pure. Silica-gel films were prepared as follows. Silica-gel (5 g) was suspended in a 2% aqueous solution of sodium borate (10 ml) and sprayed on glass plates (20 pieces 25 mm × 70 mm). Pink spots were developed by spraying of sulfuric acid and by gentle heating. The trans isomer is more mobile than the cis isomer.

3′,4′,5′,5,7-Pentamethoxyflavan-3,4-diols. The cis and trans isomers were prepared by the procedure described above. The cis isomer separated from methanol as needles, mp 195—196°C, and showed the one spot on a thin-layer chromatogram. However, the trans isomer, recrystallized repeatedly from methanol and benzene (as needles, mp 183—184°C), showed two spots. The lower spot corresponded to the spot of the cis isomer.

3′,4′,5′,5,7-Pentamethoxy-3-hydroxyflavanone-4-^{18}O. To a solution of 3-hydroxyflavanone (2.5 g) in 140 m*l* of dioxane there were added 10 m*l* of heavy water (^{18}O; 5.2%) and a trace of hydrochloric acid, and the solution was kept at 30°C for 2 days. After the removal of the solvent under reduced pressure, the remaining white crystals were separated from methanol as needles, mp 194.5—195.5°C, 2.0 g. The analysis of oxygen-18 showed an ^{18}O content of 5.1%, which was calculated from the $M+2/M$ ratio of CO_2 (^{18}O; 5.4%, calculated from $M+2/M$ ratio of the flavanone-4-^{18}O).

Analysis of Oxygen-18. The mass spectra of flavan-3,4-diols and isopropylidene derivatives were measured with a Hitachi RMU-6D mass spectrometer and a direct-inlet system, operating with an ionization energy of 70 eV. The $M+2/M$ ratio was determined from the intensities of peaks by slow scanning and was corrected by considering the incorporated deuterium atoms.

To analyze oxygen-18 in the form of carbon dioxide, samples were decomposed with mercuric cyanide and mercuric chloride in a vacuum, according to the method of Rittenberg and Ponticorvo.[6]

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