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Dynamic NMR investigation of two new interconvertible diasteriomeric epimers of natural 2-benzyl-2-hydroxybenzofuranone derivative from *Pterocarpus marsupium*☆,☆☆

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Abstract—The first example of a pair of interconvertible diastereomeric epimers 2α/2β-hydroxy-2-*p*-hydroxybenzyl-3(2*H*)-benzofuranone-7-C-β-D-glucopyranoside isolated from the heartwood of *Pterocarpus marsupium* is reported. The predominance of **1a** over **1b** was supported by dynamic exchange rates and activation parameters obtained from NMR studies. The mechanism of this unique phenomenon is thought to be operative by the formation of diketone as suggested by deuterium exchange.

1. Introduction

Wooden tumbler made up of heartwood of *Pterocarpus marsupium* also known as Indian kino or Bijay sar is used for drinking water as a traditional remedy in India because of its medicinal property[1,2] and notably for controlling blood sugar level. The plant is reported to be rich in polyphenolic compounds.[3] To determine the chemical basis for traditional cures, we have isolated constituents from the aqueous extract of the freshly prepared wooden tumbler as described in Section 4. Herein, we report a new pair of exchangeable diastereomeric epimers **1a** and **1b** which are interconverting so readily that they exist as an inseparable, equilibrium mixture at room temperature.

2. Results and discussion

2.1. NMR analysis and structural determination

The diastereomeric marsuposide **1a** and **1b**, (2α/2β-hydroxy-2-*p*-hydroxybenzyl-3(2*H*)-benzofuranone-7-C-β-D-glucopyranoside), gave the molecular composition as $C_{21}H_{22}O_{10}$ (FAB-MS, *m/z* 435 $[M+H]^+$), and was supported by ^1H and ^{13}C NMR spectroscopic data. The ^1H NMR indicated the material as a mixture of two epimeric diasteromeric compounds. Unfortunately, these proved to be inseparable by HPLC using several solvent combinations. ^{13}C NMR along with DEPT spectra provided further evidence for the presence of two isomeric compounds, showing four similar chemical shifts in their 18-carbon system. The ^1H NMR in CD_3OD indicated the presence of two anomeric protons at 4.71 and 4.75 ppm, along with other sugar and aliphatic resonances between 2.90 and 4.80 ppm. In the aromatic region two sets of *ortho* coupled aromatic protons at 6.59, 7.13 and 6.59, 7.03 ppm and a pair of A_2B_2 system at 6.47, 7.28 and 6.46, 7.29 ppm, respectively. The integral of two distinct signals at 7.03 and 7.13 ppm suggested the ratio of the diastereomers as 1.00:0.78. The complete structure determination was carried out by the combination of COSY, HMQC and HMBC (See Supporting information) spectra. The COSY cross peaks correlation was straight forward, having two sets of *ortho*-coupled spin system in the aromatic region. In HMQC spectra, the anomeric protons correlated with a carbon at 75.1 ppm, characteristic of C_1-substituted glucosides. The starting point for the assignment and evaluating the positions of the various functional moieties in the benzofuranone system was the benzylic methylene proton which appeared as two sets of AB quartets at 3.08, 3.11 and 3.02, 3.11 having a geminal coupling of 13.7 Hz.

☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2004.01.012

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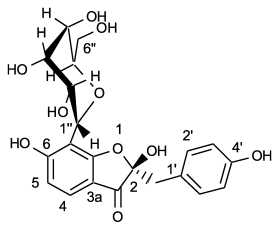


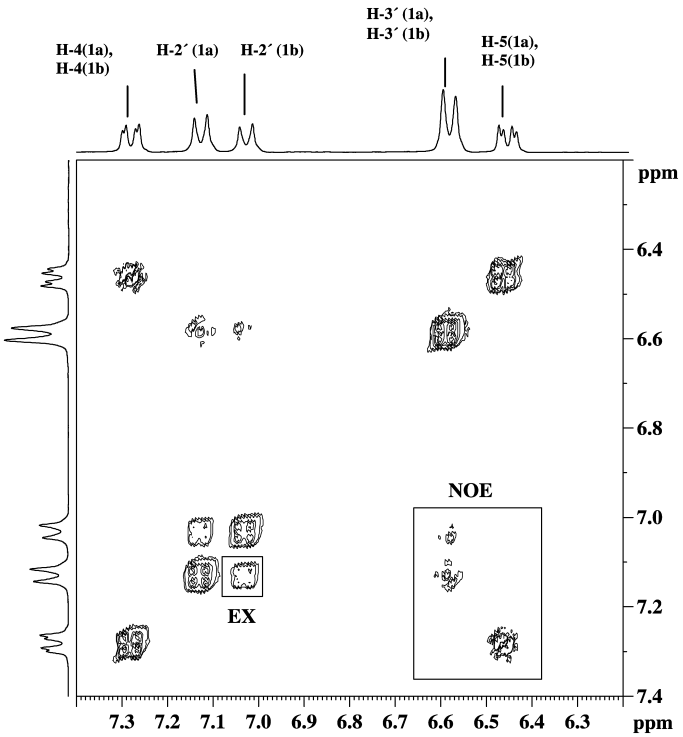
1a

1b = C-2 epimer

In the HMBC spectrum these protons showed cross peaks with the carbonyl carbon of the benzofuranone at 199.7 ppm, the aromatic CH carbons of the phenyl ring (125.8, 133.2, 132.7 ppm) and with the quaternary carbons at 107.6 and 107.8 ppm, respectively. These quaternary carbons were assigned to be C-2 of the benzofuranone ring having hydroxyl and benzylic phenol as substituents. The point of attachment of the C-glucose and a hydroxyl function furan was relatively straightforward as H-1″ of the glucose gave cross peaks with the sets of carbons at (107.8, 107.6), (168.1, 168.0) and (173.3, 173.2) ppm in the HMBC corresponded to the C-7, C-6 bearing hydroxyl function and C-7a, respectively. Thus specific and detailed ^{1}H and ^{13}C assignments of both the isomers could be differentiated from HMBC cross peak correlations and is provided in Section 4. The relative stereochemistry at C-2 could not be defined, even with ROESY and HSQMBC.[4] Moreover, an unusual exchange positive cross peak between H-2′ of **1a** and **1b** was observed in the ROESY spectrum. This prompted us to re-investigate its internal dynamics by two-dimensional exchange spectroscopy (NOESY) in deuterated methanol, and acetonitrile. Cross peaks appear with a positive sign (Fig. 1) between the corresponding protons of different epimers (Ex), whereas NOE peaks appear as negative sign between different protons of the same epimer. The interconversion was also observed in deuterated acetone at room temperature as found from the 2D exchange spectroscopy and in DMSO-d_6 at 353 K, respectively. The higher temperature required in DMSO-d_6 can be attributed to the formation of hydroxy–DMSO complex[5] and hence stabilizing the cyclic hemiacetal form, which prevents the interconversion at room temperature. Whereas, weak intermolecular hydrogen bond interaction may not restrict the interconversion in case of acetone-d_6.

Further, it is pertinent to point out here that in the case of maesopsin and related bioflavonoid, reported by Ferreira[6] and co-workers stated the complexity of the resulting fraction as mixtures by ^{1}H NMR spectrum and proposed derivatization being prerequisite for sample purity, suggested that this was consistent with the existence of epimeric mixtures. Methylation of the compound resulted in the separation of the epimeric diasteromers using FCC by the above-mentioned workers, indicated methylation stops the interconversion.

With due course of time, change in the ^{1}H and ^{13}C NMR pattern of benzylic methylene (CH_2) Figure 2(a) was observed in CD_3OD thus indicating the incorporation of deuterium, respectively. This was further confirmed by ^{2}H NMR, FAB-MS and ES-MS. ^{1}H NMR integration ratio showed 75% of total deuterium incorporation. Whereas in ES-MS the break up percentage of native form, one deuterated incorporated and two deuterium incorporated at the benzylic position was found to be 9.6, 39.4 and 50.9%, respectively. The important feature of the ^{2}H NMR spectra Figure 2(b) was the observation of a broad peak between



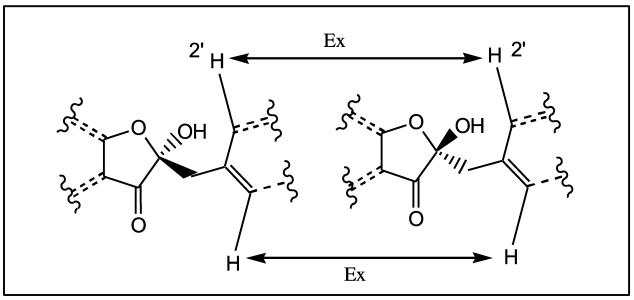


Figure 1. Portion of the NOESY spectrum showing the exchange cross peaks amongst epimers in CD_3OD at 298 K (mixing time of 400 ms).

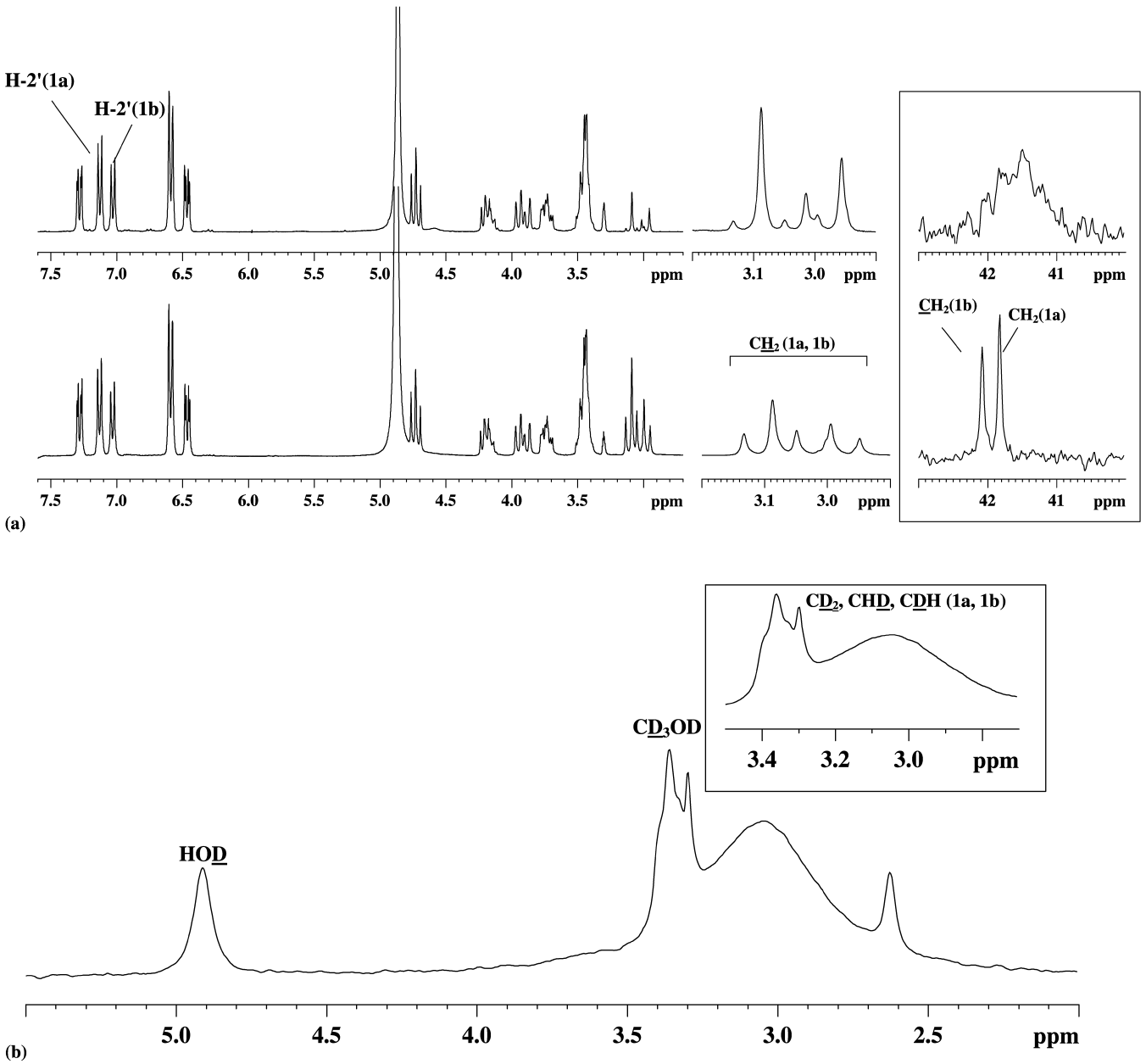


Figure 2. (a) ^{1}H and part of ^{13}C NMR in CD_3OD before (below) and after (above) deuterium exchange. (b) ^{2}H NMR in CH_3OH after deuterium exchange.

2.90 and 3.20 ppm along with other expected signals representing different deuterated species. No peaks were observed beyond δ 2.0 and 5.5 ppm. Furthermore, the ES-MS in CH_3OH showed peaks at *m/z* 435, 436 and 437 and in CD_3OD at *m/z* 464, 465 and 466 $[M+7+Na]^+$ corresponding to **1a** or **1b** in native form, **1a** or **1b** with one deuterium incorporated, **1a** or **1b** with two deuterium incorporated depicting the presence of sets of isomers. Seven corresponds to the number of exchangeable –OH protons in CD_3OD (Supporting information). In order to define the relative stereochemistry at C-2, CD spectrometry[6,7] was carried out. The CD spectra (Fig. 3) of the mixture of epimers provided the resultant spectrum, since the racemization will lead to proportional diminishing of the transition.[8]

Negative amplitude cotton effect for $\pi\rightarrow\pi^*$ transition at 320 nm and positive amplitude cotton effect for $n\rightarrow\pi^*$ transition at 350 nm of the CD spectra[6,7] suggested 2*R* (**1a**) is in excess with 2*S* (**1b**), respectively, and is in accord with the ^{1}H NMR spectrum. To establish the kinetic order character of the exchange process, the concentration dependence of the NOESY spectra has been studied. We found no change in the intensity of the peak upon changing the 55.3 mM concentration to half at 298 K with a mixing

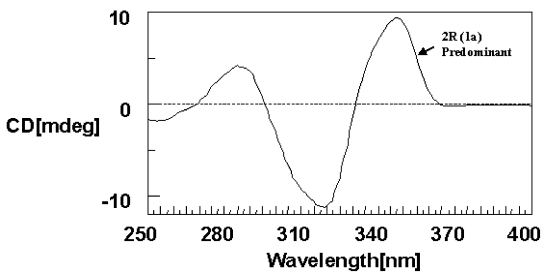


Figure 3. Resultant CD spectra of marsuposides **1a** and **1b**.

time of 400 ms. Under these conditions, the exchange was about 22% complete, and a substantial change in the rate constants would have been immediately reflected in the intensity of the exchange peak for a higher order reaction. It was concluded that the exchange process follows first-order kinetics. The rate of the first order kinetics was calculated by performing phase sensitive 2D-NOESY using 200, 300 and 400 ms as mixing time at five different temperatures; 293, 298, 303, 313 and 318 K in CD_3OD. The values of the rate constants have been obtained by Eq. 1[9] and are presented in Table 1.

Table 1. Rate constants determined by using Eq. 1[9]

T/K	293	298	303	313	318
k_1 (s^{-1})	0.38±0.01	0.51±0.06	0.69±0.12	1.29±0.34	1.54±0.49
k_{-1} (s^{-1})	0.47±0.02	0.61±0.12	0.76±0.20	1.52±0.38	1.71±0.75

$$K \simeq 1/[t_M(I_D/I_C + 1)] \quad (1)$$

Similarly, rates were calculated in CD_3CN using 200, 300 and 400 ms mixing times at five different temperatures; 268, 273, 278, 283 and 288 K (Table 2).

Table 2. Rate constants determined by using Eq. 1[9]

T/K	268	273	278	283	288
k_1 (s^{-1})	0.32±0.07	0.46±0.13	0.68±0.22	1.01±0.22	1.35±0.54
k_{-1} (s^{-1})	0.42±0.03	0.58±0.10	0.84±0.22	1.23±0.20	1.61±0.45

The temperature chosen in case of CD_3CN was based on the fact that at 298 K the peaks were broad due to the presence of fast exchange between **1a** and **1b**. At temperatures lesser than 293 K, good separation of H-2′ signal of **1a** and **1b** was observed.

Notably, different rates were observed in $(CD_3)_2C{=}O$ and DMSO-d_6 depicting the effect of the solvent. Since the reaction rate constants have been determined at different temperatures in CD_3OD and CD_3CN an estimation of the activation parameters were possible using the Eyring's equation (Eq. 2).[10]

$$\ln(k/T) = 23.76 - (-\Delta H^{\ddagger}/RT) + (\Delta S^{\ddagger}/R) \quad (2)$$

The straight line in the Erying plot (Figs. 4 and 5) suggested that the two rate constants have the real physical meaning rather than just being fit parameters, thereby adding credibility to the exchange process.

The activation parameters determined from Eyring plot are given in Table 3. With regard to mechanistic interpretation,

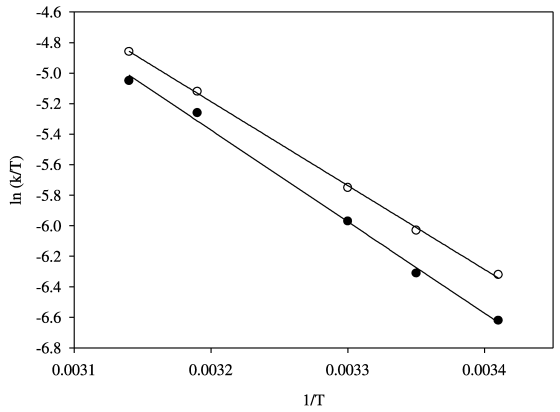


Figure 4. Eyring plot of the rate constants in CD_3OD with 200 ms mixing time determined by fitting the rate constants k_1 and k_{-1} to the experimental data at different temperatures.

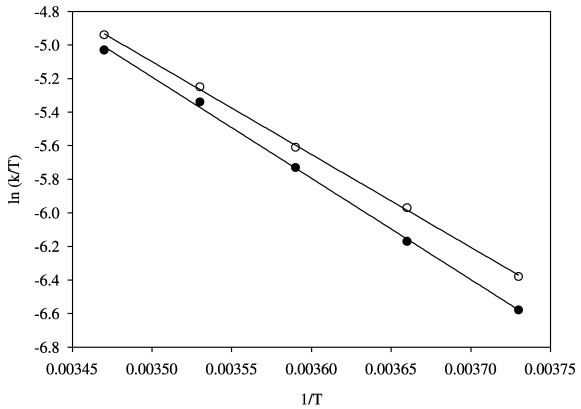


Figure 5. Eyring plot of the rate constants in CD_3CN determined with 200 ms mixing time by fitting the rate constants k_1 and k_{-1} to the experimental data at different temperatures.

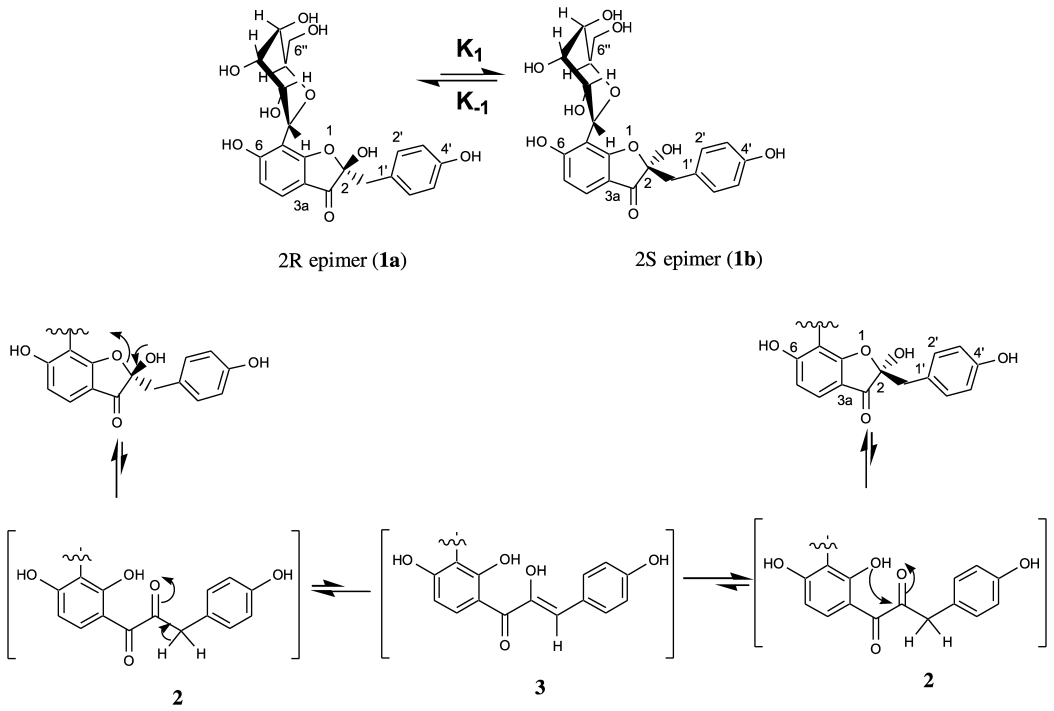
a rationalization is given in Scheme 1 involving **2** as a conceivable intermediate which converted into the enol form. Hence, the key pathway for the deuterium incorporation in CD_3OD was envisaged to be keto enol tautomerism (Scheme 2).

Consistent with this proposal, the $\Delta H^{\ddagger}$ of isomerization clearly suggests that **1a** would be more populated than **1b**, as borne out from the NMR measurements (Tables 1 and 2). However, no characteristics signals in ^1H and ^{13}C NMR spectra were observed in our efforts to trace the intermediates diketo **2** and enol form **3** at the lowest possible temperature of 223 K in CD_3OD and 233 K in CD_3CN. This suggested that the relative concentration of **2** is lower than the NMR dynamic range (NMR detection limit) and for **3** the equilibrium lies well over towards the keto form. Hence the experimental evidence for **2** and **3** could not be achieved.

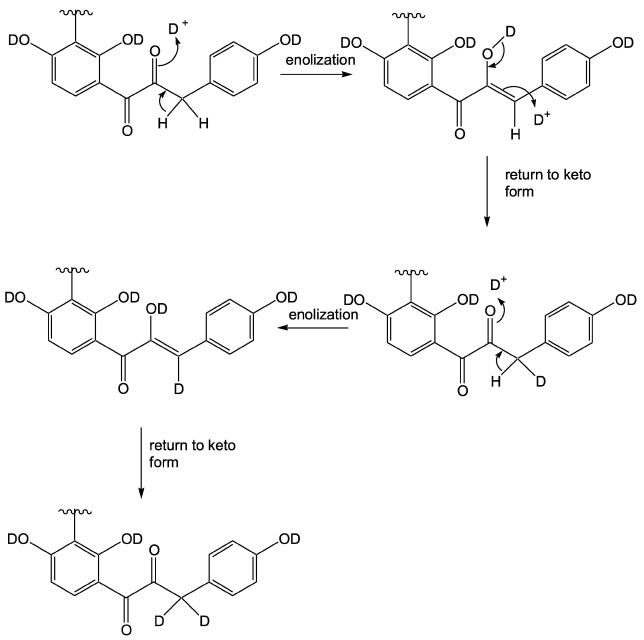
Table 3. Activation parameters obtained from Eyring analysis by using Eq. 2 for rate constants given in Tables 1 and 2[a]

	CD_3OD		CD_3CN	
	k_1	k_{-1}	k_1	k_{-1}
$\Delta H^{\ddagger}$ (kJ/mol)	42.0±7.4	39.5±10.2	44.9±15.1	41.7±10.1
$\Delta S^{\ddagger}$ (J/mol K)	−107.3±25.4	−114.2±34.0	−83.8±48.1	−93.6±43.1

[a] The indicated standard errors of regression take into account the error margins of the individual rate constants.



Scheme 1. Mechanism of interconvertibility.



Scheme 2. Proposed mechanism of deuterium incorporation.

3. Conclusion

In summary, we have demonstrated the exchange between new diastereomeric epimers 2α/2β-hydroxy-2-*p*-hydroxybenzyl-3(2*H*)-benzofuranone-7-C-β-D-glucopyranoside by NMR exchange spectroscopy (NOESY) at different temperatures in different solvents. The first order rates confirmed 2*R* to be more populated. The mechanism of exchange involves ring opening to form diketone followed by keto enol tautomerism which was evident by deuterium incorporation in CD_3OD as studied by ^2H NMR and ES-MS. Due to the exchange property of the molecule, attempts to isolate the individual native diastereomeric epimers at room temperature are unlikely to succeed even using chiral columns.

4. Experimental

4.1. Extraction

The freshly prepared wooden tumbler was purchased from the local Indian market. It was crushed, powdered and was exhaustively extracted with hot water (4×16 ml). The concentrated, extract (500 g) was suspended in H_2O (2.0 l) and successively partitioned with EtOAc and *n*-BuOH. Part (65 g) of the *n*-BuOH extract (170 g) on repeated flash chromatography over silica gel using $CHCl_3$–MeOH (9:1) as solvent, and HPLC separation afforded marsuposide (**1a/1b**, 50 mg).

4.2. General procedure

Melting points was recorded with melting point apparatus and is uncorrected. IR spectra were recorded with FTIR spectrometer (Perkin–Elmer RXI) as a KBr pallet (expressed in cm^{-1}). ^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded in CD_3OD, CD_3CN, $(CD_3)_2CO$ and DMSO-d_6 (Aldrich) as solvent on a Bruker Avance DRX-300 calibrated with TMS. ^2H NMR (46 MHz) was recorded in CH_3OH as solvent in unlock mode by online shimming of the sample using interactive 1D ^1H NMR spectrum. NMR chemical shifts determined from HMQC and HMBC data are reported in δ (ppm) were referenced to TMS (0.0 ppm) for proton and carbon. Optical rotation was measured with a polarimeter (Rudolf Autopol III) using sodium light (D line 589.3 nm) at 25 °C. CD data were recorded in MeOH on a JASCO J-710 spectropolarimeter at

25 °C. ES-MS was recorded in CD_3OD and CH_3OH on a Micromass Quattro II. The FAB-MS was recorded using a Jeol SX-120/DA6000 mass spectrometer using Ar as the FAB gas. TLC was performed on a precoated Merck Aluminium sheets (silica gel 60 PF_{254}) and the column chromatography was performed on 200–400 mesh silica gel.

Marsuposide, light yellow crystalline, mp 156–158 °C, $[\alpha]_D^{26}$ +8.4° (*c* 0.225, CH_3OH) IR (KBr, $\nu\,cm^{-1}$) 3300, 1680, 1608, 1510, 1444.

4.2.1. Compound 1a. ^1H NMR (300 MHz, CD_3OD): 3.11 (1H, d, *J*=13.9 Hz, CH_{2a}), 3.08 (1H, d, *J*=13.9 Hz, CH_{2b}), 7.28 (1H, d, *J*=8.4 Hz, H-4), 6.47 (1H, d, *J*=8.4 Hz, H-5), 7.13 (1H, d, *J*=8.4 Hz, H-2′), 6.59 (1H, d, *J*=8.4 Hz, H-3′), 4.75 (1H, d, *J*=10.3 Hz, H-1″), 4.20 (1H, m, H-2″), 3.44 (1H, m, H-3″), 3.45 (1H, m, H-4″), 3.45 (1H, m, H-5″), 3.11(1H, dd, *J*=12.1, 2.2 Hz, H-6″a), 3.76 (1H, dd, *J*=10.3, 5.9 Hz, H-6″b). ^{13}C NMR (75 MHz, CD_3OD): 107.6 (C-2), 199.7 (C-3), 41.8 (CH_2), 113.3 (C-3a), 126.8 (C-4), 112.8 (C-5), 168.1 (C-6), 110.3 (C-7), 173.4 (C-7a), 125.8 (C-1′), 133.2 (C-2′), 115.9 (C-3′), 157.3 (C-4′), 75.0 (C-1″), 72.5 (C-2″), 80.3 (C-3″), 72.4 (C-4″), 82.7 (C-5″), 63.7 (C-6″).

4.2.2. Compound 1b. ^1H NMR (300 MHz, CD_3OD): 3.11 (1H, d, *J*=13.9 Hz, CH_{2a}), 3.02 (1H, d, *J*=13.9 Hz, CH_{2b}), 7.29 (1H, d, *J*=8.4 Hz, H-4), 6.46 (1H, d, *J*=8.4 Hz, H-5), 7.03 (1H, d, *J*=8.4 Hz, H-2′), 6.59 (1H, d, *J*=8.4 Hz, H-3′), 4.71 (1H, d, *J*=10.3 Hz, H-1″), 4.10 (1H, m, H-2″), 3.44 (1H, m, H-3″), 3.45 (1H, m, H-4″), 3.45 (1H, m, H-5″), 3.88 (1H, dd, *J*=12.1, 2.2 Hz, H-6″a), 3.71 (1H, dd, *J*=10.0, 4.8 Hz, H-6″b). ^{13}C NMR (75 MHz, CD_3OD): 107.8 (C-2), 199.7 (C-3), 42.1 (CH_2), 113.2 (C-3a), 126.8 (C-4), 113.0 (C-5), 167.9 (C-6), 109.9 (C-7), 173.3 (C-7a), 125.8 (C-1′), 132.7 (C-2′), 116.0 (C-3′), 157.4 (C-4′), 75.2 (C-1″), 72.7 (C-2″), 80.3 (C-3″), 71.8 (C-4″), 82.7 (C-5″), 63.1 (C-6″).

FAB-MS; *m*/*z* 435.0 $[M+H]^+$. Anal. calcd for $C_{21}H_{22}O_{10}$: C, 58.06; H, 5.10. Found C, 57.99; H, 5.04%.

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