

stirred. After workup, an oily residue **8**, (70 mg, 66.3%) was obtained.

Dehydration of 2-(N-acetylaminoethylthio)acetoacetanilide enol **8**

A solution of 2-(N-acetylaminoethylthio)acetoacetanilide enol **8** (1.0 g, 3.4 mmol) and *p*-toluenesulfonic acid (32 mg) in benzene (250 ml) was refluxed for 25 h with a Dean-Stark water trap. The reaction mixture was cooled, washed with cold water and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give an yellow foamy residue (640 mg). Crystallization from ethyl acetate and petroleum ether gave **1a** (490 mg, 52%) as a light yellow needle, mp. 91-93°C; ¹H-NMR (300 MHz) (CDCl₃) δ 2.17 (s, 3H, CH₃CO), 2.40 (s, 3H, 3-CH₃), 3.18 (t, 2H, *J*=6.0 Hz, 6-CH₂S), 3.79 (t, 2H, *J*=6.0 Hz, 5-CH₂N), 7.15 (t, 1H, *J*=8.1 Hz, *p*-ArH), 7.35 (t, 2H, *J*=7.5 Hz, *m*-ArH), 7.57 (d, 2H, *J*=7.7 Hz, *o*-ArH), 8.35 (br, s, H, NH).

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- Because the chlorine solution in methylene chloride used in chlorinolysis could not be dried absolutely (see the experimental section), the small amount of water was in the reaction medium.
- It is interesting that we observed only the enol form **8** at room temperature although the keto form **20** should be possible in equilibrium in most cases⁷.
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The Application of NMR Techniques to the Structural Confirmation of *O*-Substituted 3,4-Dihydroxyphenylacetic Acid Derivatives

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The structures of the compounds, **1**, **2**, **3**, and **4**, which were precursors of analgesics, were confirmed by modern NMR techniques. The complete ¹³C-NMR assignments of these systems were established by applying COLOC (COrelated spectroscopy for LONG range Couplings), HETCOR (HETeronuclear COrelated spectroscopy), RCT (Relay Coherence Transfer), and NOE difference spectroscopy. The limitation of COLOC approach which has been widely used recently is discussed.

Introduction

Capsaicinoid is a pungent principle of red pepper.¹ Red pepper has been used as food additives and folk medicine for a long time. Capsaicinoid was found to be responsible for analgesic activity. Structural elucidation by Bennett and Kirby concluded that it was a mixture of at least five analogous amides including capsaicin [*N*-(4-hydroxy-3-methoxybenzyl)-*trans*-8-methyl-6-nonenamide].²

In the course of our continuing study on analgesic activity of capsaicinoids,³ we became interested in the synthesis of 3-hydroxy analogs. The synthesis required partial alkylation of phenolic hydroxy group of 3,4-dihydroxyphenylacetic acid derivatives. Unfortunately, selective demethylation of 4-al-

koxy-3-methoxyphenylacetamide was not successful. No selectivity was observed when monobenylation was attempted and the products were separated by MPLC.⁴ In confirming the substitution patterns, the ¹H-NMR spectra were not distinguishable because of the inherently low dispersion of ¹H substituent chemical shift as it can be predicted. Although ¹³C-NMR spectroscopy has been used to determine the structures of substituted aromatic systems based on theoretical treatment and empirical data, the former still has the lack of flexibility and restriction on the range of application and the latter has the limited tabulations. Furthermore, signal assignment between hydroxylated and *O*-alkylated carbons based on chemical shift calculations is not reliable because the substituent effect of OH, OR¹, and OR² groups are very

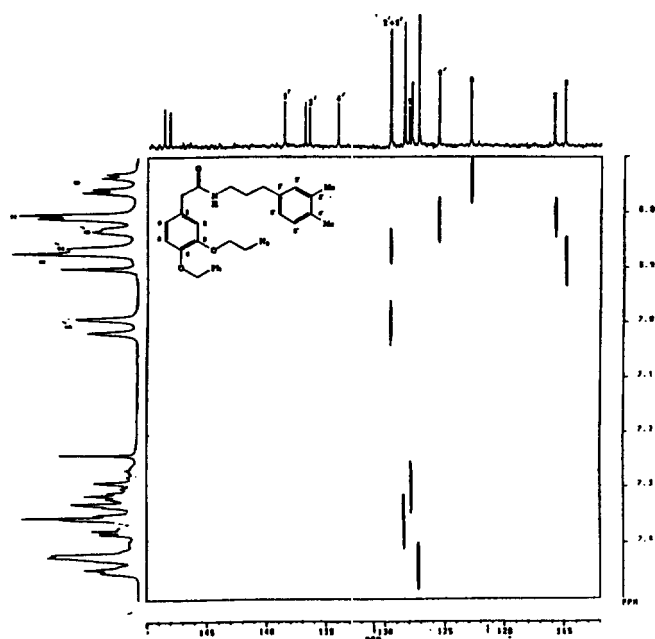
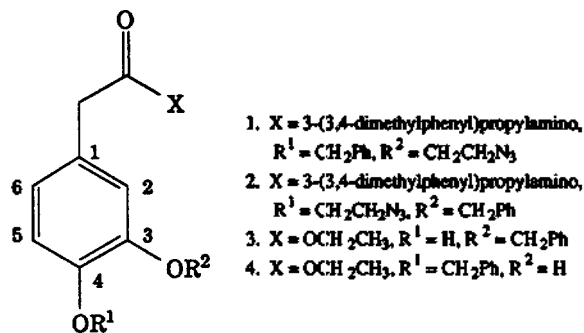


Figure 1. Partial 2D C-H correlation spectrum of compound 1.

comparable.⁵ Therefore, the structures of these compounds have been determined by an independent synthesis as will be reported elsewhere.⁴



However, throughout the last decade, the development of modern high-resolution NMR spectroscopy, which has enabled the complete or nearly complete stereochemical and conformational analysis for a variety of molecules,⁶ would be a very useful method to carry out this task. Particularly scalar and dipolar ¹H-¹H interactions at high magnetic fields and two-dimensional chemical shift correlation are most useful methods in this regard. Recently, Huber and Parker reported a structural determination of partially *O*-methylated phenolic biphenyl compound by using heteronuclear chemical shift correlation (HETCOR), spin-lattice relaxation time, deuterium isotope effect, and NOE difference spectroscopy.⁷

We report here the results of structural assignment of *O*-substituted 3,4-dihydroxyphenylacetic acid derivatives **1**, **2**, **3**, and **4** on the basis of two-dimensional HETCOR,⁸ correlated spectroscopy for long range coupling (COLOC),⁹ relay coherence transfer (RCT),¹⁰ and NOE difference spectroscopy.¹¹

Results and Discussion

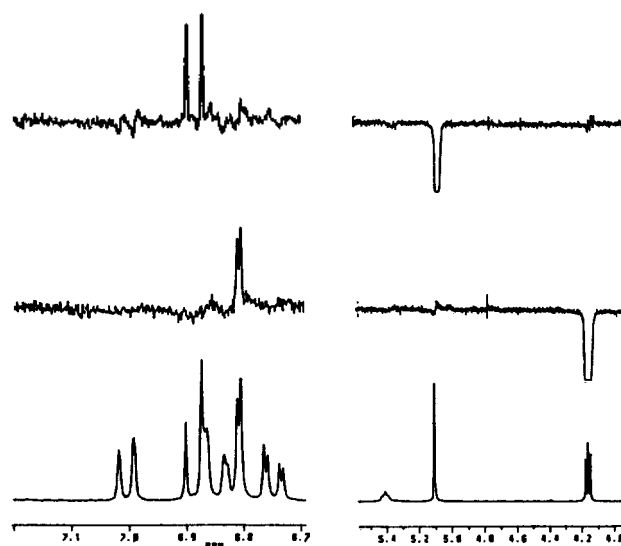


Figure 2. NOE difference spectra of compound 1.

The first task of this study was to assign the ¹H and ¹³C peaks of the highly congested aromatic region as much as we could. Partial assignments of the proton resonances could be made with a reasonable degree of confidence on the basis of conventional chemical shift and coupling constant arguments. Protons **2**, **5**, and **6** in the compound **3** and **4** were easily assigned at upfield (relative to unsubstituted benzyl group protons) on the basis of substitution effect and coupling patterns (*vide infra*). However, the protons **2**, **5**, and **6** in **1** and **2** were overlapped with the protons of 3,4-dimethylphenyl moiety. The aromatic region of the two-dimensional C-H correlation spectrum of **1** is shown in Figure 1 along with the one-dimensional ¹H and ¹³C spectra. Although the partial assignment of ¹³C spectra could also be made with the substitution effect and DEPT experiment,¹² the correlation peaks in Figure 1 clearly differentiate the chemical shifts of quaternary carbons. In this spectrum quaternary carbons did not show correlation peaks because the optimum polarization time ($1/2 J$) and refocusing time ($1/4 J$) were adjusted to one bond C-H coupling (140 Hz). This result and DEPT experiment led to the conclusion that the carbon resonance at 129.6 ppm represented two methylene carbons. Consequently, the proton multiplet peaks at 7.1-6.7 ppm were responsible for six protons.

The prediction that proton **6** is observed at higher field relative to proton **6'** based on substitution effect is also confirmed by the results of NOE difference and RCT experiments. As an alternative method, NOE approach enables a self-consistent structural analysis by only the assignment of ¹H-NMR spectra. This mechanism is inversely related to the sixth power of the interproton distance and therefore NOE effect decreases quickly as the interproton distance increases. The results of NOE difference experiments of compound **1** (*vide infra*) are shown in Figure 2 along with normal proton spectrum. When the methylene protons in R¹ are irradiated, a 2.4% NOE enhancement is observed at 6.89 ppm as a doublet ($J=8.2$ Hz). However, the irradiation of *O*-methylene protons in R² produces a 2.2% of NOE enhancement at 6.81 ppm as a doublet ($J=1.8$ Hz). These results and coupling

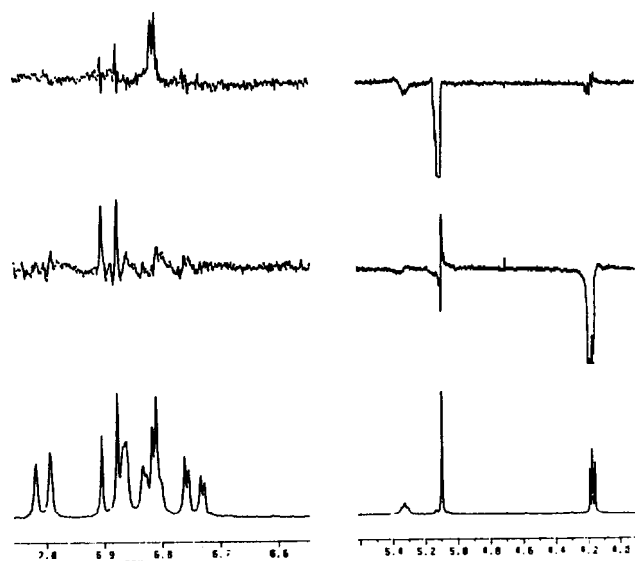


Figure 3. NOE difference spectra of compound 2.

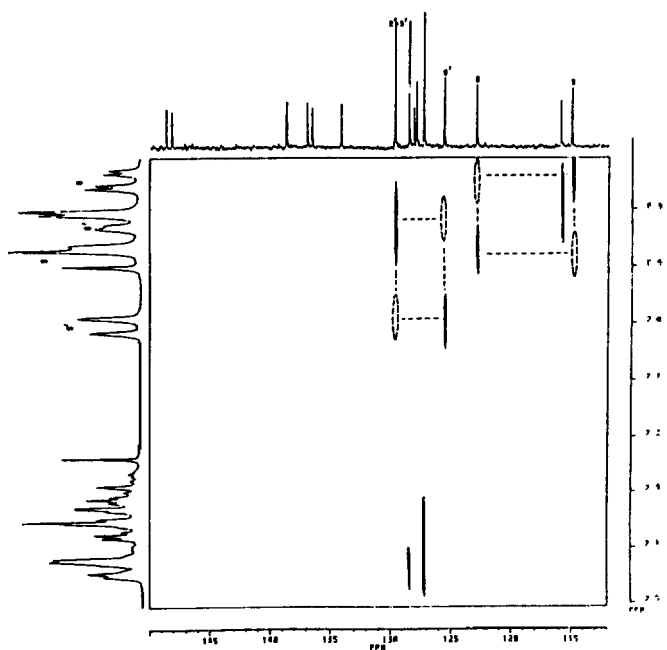


Figure 4. Partial Relay Coherence Transfer Spectra of Compound 1.

constants consideration enable the assignment of 2 and 5 protons. This assignment is also supported by the NOE difference experiment in the compound 2. Thus, the irradiation of the methylene protons in R^2 yields a 2.6% NOE effect in proton 2 and the irradiation of the *O*-methylene protons in R^1 produces a 3% NOE enhancement in proton 5 (Figure 3).

By analogy, the protons 2' and 5' could be distinguished. The protons 5 and 5' identified by previous ways could be used to confirm the protons 6 and 6' in the RCT experiment. This H-C relay experiment is useful in providing informations about protons that are vicinally coupled to a proton attached to a given carbon atom. Thus, when Figure 4, which is RCT spectrum of compound 1, is compared with Figure

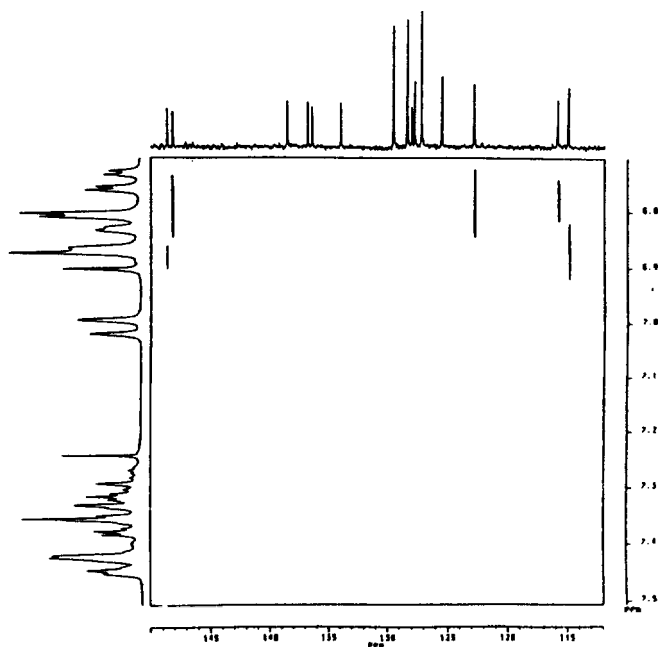


Figure 5. COLOC Spectrum of Compound 1.

The data were acquired with $J=7$ Hz and $4\text{ K}\times 160$ data points and followed by zero-filling to give $4\text{ K}\times 512$ data matrix.

1, the cross peaks clearly show the connectivities of 5-6 and 5'-6' (dashed ellipses are the positions of the missing signals from direct H-C connectivity).

In experiments described thus far, we could not assign the quaternary carbons. Finally, we describe here the application of long range H-C coupling in compounds 1, 2, 3, and 4, because the assignment of quaternary carbons is another way of distinguishing these isomers. Recently, a growing number of studies have utilized long range heteronuclear coupling pathways (${}^nJ_{\text{CH}}$, $n=2, 3$) to determine the molecular structures.¹³ In order to do this experiment, we applied COLOC pulse sequence developed by Kessler and Griesinger. To contemplate this experiment, knowledge on the size of the various long range heteronuclear coupling constants must be available. Unfortunately, compilations of heteronuclear coupling constants are far fewer in number than those of homonuclear coupling constants. In general, the other option is to optimize the coupling constants by using a set of survey parameters. In our cases, there are two possible parameter selections. Thus, one option is to apply two-bond couplings, the other is to use three-bond couplings. Especially, because the differentiation of carbons 3 and 4 is very important (*vide supra*), the protons 2 and 5 seem to be very useful to correlate the carbons 3 and 4, respectively. However, it is impractical to optimize the experiment for small couplings because of the losses of signal that will occur during the long defocusing ($1/2 J$) and refocusing ($1/4 J$) delays. Therefore, we used here three-bond couplings to correlate the protons 2 and 6 for the carbon 4 and the proton 5 for the carbon 3. Generally, three-bond coupling constants are known to be in the range of 5 to 12 Hz.¹⁴

Representative COLOC spectrum of compound 1 is recorded with $J=7$ Hz and shown in Figure 5. In this spectrum, the quaternary carbon at 148.64 ppm has correlation peak

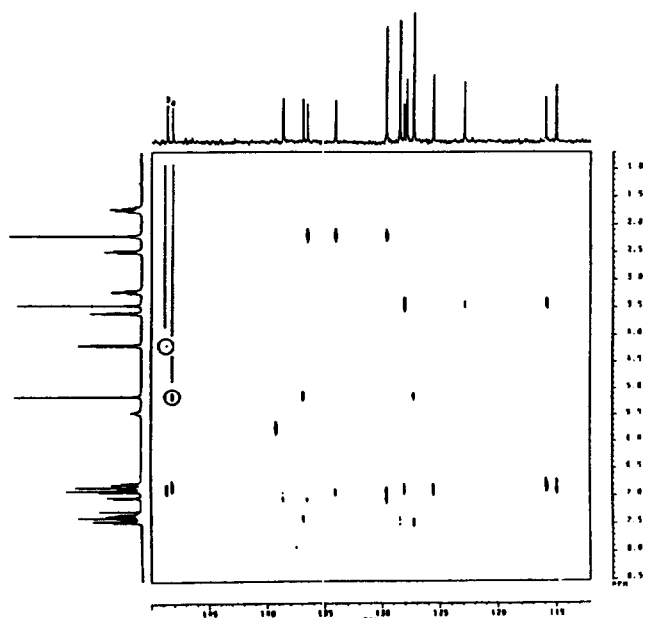


Figure 6. COLOC Spectrum of Compound 1. The data were acquired with $J=9$ Hz and $4\text{ K}\times 128$ data points and followed by zero-filling to give $4\text{ K}\times 256$ data matrix.

Table 1. ^{13}C Chemical Shifts of Phenyl Ring Carbons

Compound	Carbon Number					
	1	2	3	4	5	6
1	128.07	115.91	148.64	148.18	115.02	122.88
2	128.79	115.76	149.14	147.67	115.29	122.24
3	125.75	113.09	145.67	144.89	114.57	122.41
4	127.60	115.70	145.73	144.86	112.13	120.66

with proton 5 and the slightly upfield carbon at 148.18 ppm has correlation peaks with protons 2 and 6, respectively. Therefore, the carbon 3 chemical shift is assigned downfield shift relative to that of the carbon 4 in the compound 1. This assignment is also supported by the fact that the benzylic methylene proton in R^1 has a correlation peak with the carbon 4 and *O*-methylene proton in R^2 has a correlation peak with the carbon 3 in Figure 6 recorded with $J=9$ Hz. All of these methods mentioned previously enable the complete assignment of the spectrum of compounds 1, 2, 3, and 4 and the six carbon chemical shifts are listed in Table 1. As can be seen in Table 1, the chemical shift of carbon 3 is always observed at down field relative to that of carbon 4 in all of these four compounds. In compound 3, the chemical shift of carbon 2 is observed at higher field relative to the that of carbon 5.

Although the COLOC spectrum can be used as an assignment technique as described previously with the other techniques, its limitation can be seen in Figure 5 which shows a poor resolution in F1 dimension. Thus, the correlation peaks of the protons 2 and 6 with carbon 4 are overlapped. In this pulse sequence, the sweep width and digitization in F2 frequency domain can be freely selected. However, in F1

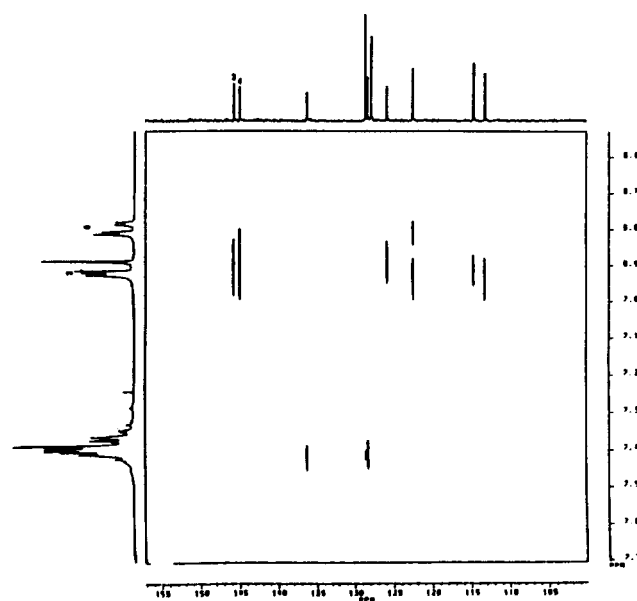


Figure 7. COLOC Spectrum of Compound 3 at 300.13 MHz. The data were acquired with $J=7$ Hz and $4\text{ K}\times 160$ data points and followed by zero-filling to give $4\text{ K}\times 512$ data matrix.

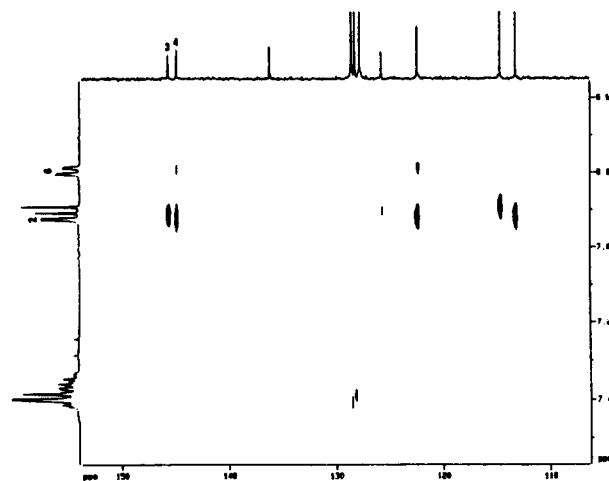


Figure 8. COLOC Spectrum of Compound 3 at 500.13 MHz. The data were acquired with $J=7$ Hz and $4\text{ K}\times 160$ data points and followed by zero-filling to give $2\text{ K}\times 512$ data matrix.

domain it is not possible in all cases to employ the desired sweep width and the level of digitization. If we desire to maintain the optimization on large coupling constant, we must either increase the sweep width and/or decrease the number of increments (blocks) of data to be taken which are the factors to degrade the F1 dimension on the final spectrum.

Figure 7 is the COLOC spectrum ($J=7$ Hz) of compound 3 at 75.47 MHz (300.13 MHz at proton). In this spectrum the correlation peaks of protons 2 and 6 with carbon 4 are severely overlapped. However, those overlapped correlation peaks are clearly resolved at 125.76 MHz (500.13 MHz at proton) which is shown in Figure 8 recorded with same conditions as in Figure 7. Thus, it is worth taking note that

the application of COLOC experiment for successful assignment is limited in very congested spectrum and in low magnetic field.

In summary, the combined use of two-dimensional and NOE difference methods described here has provided a reliable protocol for the determination of stereochemistry and chemical shifts of *O*-substituted 3,4-dihydroxyphenylacetic acid derivatives.

Experimental

All spectra were recorded at ambient temperature on a Bruker AM-300 NMR spectrometer operating at 300.13 MHz for proton and 75.47 MHz for carbon except Figure 8 which was obtained on a Bruker AMX-500 spectrometer operating at 500.13 MHz for proton and 125.76 MHz for carbon. Proton and carbon chemical shifts were referenced to solvent CDCl₃. All two-dimensional spectra were recorded by using pulse programs supplied by Bruker. Both F2 and F1 dimensions were filtered by sine bell function and followed by application of magnitude calculation. NOE difference spectra were recorded by subtracting the off resonance spectra from the irradiation spectra obtained at 25°C.

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