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# DECARBOXYLATION AND EXCHANGE REACTIONS IN FLAVONOID GLYCOSIDE MALONATES

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**Key Word Index**—*Gerbera jamesonii*; Compositae; *Ipomoea tricolor*; Convolvulaceae; NMR; decarboxylation; proton–deuterium exchange; flavonoid malonylglycosides.

**Abstract**—Flavonoid malonylglycosides lose carbon dioxide and give the corresponding flavonoid acetylglycosides under conditions that are often used in determining their NMR spectra (DMSO-$d_6$ at 80–100°). If the solvent contains exchangeable deuterium the malonyl methylene protons may exchange and trideuterioacetyl derivatives form. The NMR spectra of these substances should be run initially at ambient temperature in the absence of solvents containing exchangeable deuterium.

The task of identifying and locating acyl groups attached to the sugars of plant glycosides has become almost routine since the advent of NMR spectroscopy. Acetyl and malonyl are the most common of the aliphatic acyl groups and have been reported in various naturally occurring *O*- and *C*-glycosylflavonoids, including flavones, isoflavones, flavonols and anthocyanins. In this paper we show that malonyl derivatives are apt to decompose under conditions frequently used in determining their NMR spectra.

The decarboxylation of malonic acid and its derivatives is one of the most familiar reactions of organic chemistry. Malonates are usually stable in solution at room temperature, but undergo rapid decarboxylation to the acetate as the temperature is raised to the vicinity of 100°:

$$ROCOCH_2COOH \xrightarrow{\Delta} ROCOMe + CO_2.$$

This happens to be the temperature range in which flavonoid NMR spectra are often determined, chiefly in deuterated dimethylsulphoxide (DMSO-$d_6$). It is hardly surprising then that partial or complete decarboxylation occurs during the course of the measurement. The effect is most easily seen when determining proton spectra. In one example, kaempferol 3-$\beta$-D-glucoside 6″-malonate (**1**) was dissolved in DMSO-$d_6$ and the proton spectrum obtained at room temperature. In addition to the expected aromatic and carbohydrate signals, a broad 2-proton singlet due to the malonate methylene group was observed at $\delta$3.04. The temperature of the sample was then raised to 95°. Within minutes a new signal—an acetyl methyl signal—appeared at $\delta$1.74 and continued to grow until the decarboxylation was complete (*ca* 35 min). At the same time the malonate methylene signal grew smaller and eventually disappeared entirely. Similar results were obtained with quercetin 3-$\beta$-D-glucoside 6″-malonate (**2**) and apigenin 7-$\beta$-D-glucoside 6″-malonate (**3**) (Table 1). The decarboxylation of compound **2** was usually slower than that of **1**; as much as 4.5 hr were required for the reaction to be complete. In contrast, the potassium salt of **2**, a compound found in flowers of the morning glory (*Ipomoea tricolor*), was completely decarboxylated in less than 20 minutes at 95° in DMSO-$d_6$.

The occurrence of the decarboxylation reaction is also apparent in $^{13}$C NMR spectra. The room temperature spectrum of **1** in DMSO-$d_6$ contained signals at $\delta$167.6 (COOH), 166.4 (ester C=O) and 41.1 (malonyl $CH_2$). (The malonyl $CH_2$ signal may be overlapped by solvent signals in instruments of low field strength.) The sample was then heated for 1 hr at 95° and the spectrum redetermined at room temperature. The carboxyl and methylene peaks were now absent; the ester carbonyl peak had shifted downfield to $\delta$169.6 and a new peak—the acetyl methyl peak—appeared at $\delta$20.0. Similar observations were made with **2** and **3**, as shown in Table 1.

As noted above, $^{13}$C NMR spectra are often determined in DMSO-$d_6$ at elevated temperatures. For example, in Markham and Chari's extensive review [1] about half of the 125 illustrated spectra were run at 80–100°. If the first NMR measurement taken of a malonyl derivative were a $^{13}$C spectrum at high temperature, it is conceivable that the compound might decompose and be incorrectly identified as an acetate instead of a malonate, particularly in the case of small samples that require a long period of data accumulation (and hence heating) in the spectrometer. On the other hand, larger samples requiring less instrument time, or slowly decarboxylating compounds such as **2**, would be expected to yield signals from both the malonyl and acetyl groups. We have observed the entire array of malonyl and acetyl resonances in a sample of **2** kept at 95° for 2 hr; after 4.5 hr at 95° only the acetyl resonances remained.

Further complications are likely to arise if the solvent contains exchangeable deuterium, as in DMSO-$d_6$ to which a small amount of $D_2O$ has been added in order to increase the solubility of polar compounds or reduce hydroxy proton signals and improve resolution. In this

Table 1. 1H and a3C chemical shifts (ppm) of flavonoid glucoside malonates and acetates in DMSO-d6 at room temperature

| Compound | Malonyl CH_2 | CH_3CO | Malonyl HOO_C | _CH2 | COOR | Acetyl CH3 | _COOR | Glucosyl carbon 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| KGMal (1) | 3.04 | | 167.6 | 41.1 | 166.4 | | ..... | 101A | 73.8 | 76.0 | 69.4 | 73.8 | 63.4 |
| KGAc | | 1.74 | | | ..... | 20.0 | 169.6 | 100.9 | 73.7 | 76.0 | 69.7 | 73.9 | 62.6 |
| QGMal (2) | 3.11 | | 167.5 | 40.9 | 166.3 | .... | | 101.0 | 73.9 | 76.1 | 69.5 | 73.9 | 63.5 |
| QGAc | | 1.69 | | | | 20.0 | 169.7 | 100.8 | 73.8 | 76.1 | 69.7 | 73.8 | 62.6 |
| AGMal (3) | 3.32 | ..... | 169.3<br>170.0 | * | *br* | ..... | | 99.4 | 7Z9 | 76.0 | 69.6 | 73.8 | 63.6<br>62.6 |
| AGAc | | 2.00 | -- | | | 20.4 | 169.9 | 99.4 | 72.9 | 76.0 | 69.6 | 73.7 | 63.2 |

*Abbreviations:* KGMal=kaempferol 3-fl-D-glucoside 6"-malonate; KGAc=kaempferol 3-fl-D-glucoside 6"-acetate; QGMal =quercetin 3-fl-D-glucoside 6"-malonate; QGAc=quercet!n 3-fl-o-glucoside 6"-acetate; AGMal=apigenin 7-fl-o-glucoside 6"-malonate; AGAc=apigenin 7-/~-D-glucoside 6"-acetate; R=glucosylflavonoid.

*Resonance overlapped by solvent signals.

solvent the protons of the malonyl methylene group, which is relatively acidic because of its situation between carbonyl groups, exchange with deuterium, even at room temperature.* In one experiment a sample of quercetin 3-fl-D-glucoside 6"-malonate (2) in DMSO-d 6 D20 (93:7) was kept at room temperature while its proton spectrum was being observed. After 30 min the methylene signal had decreased to about half and after 60 min to about a quarter of its original height. (As expected, the methylene signal began to show splitting as the exchange proceeded.) The sample was kept for an additional 4 hr, following which the 13C spectrum was determined. This was essentially identical to that of the original compound except for the absence of the methylene peak at 640.9. Similar results were obtained with kaempferol 3-fl-D-glucoside 6"-malonate (1), though here the exchange was more rapid [about 90% exchanged in 35 min in DMSO-d6-D20 (24: 1)]; the addition of a drop of water to the NMR tube caused partial restoration of the methylene proton peak at 63.04. When the deuterated form of compound 1 was heated at 95° it lost CO 2 and yielded 6,8-dideuteriokaempferol 3-fl-D-glucoside 6"-trideuterioacetate as indicated by NMR and mass spectral data. Proton-deuterium exchange in aromatic systems containing *meta-hydroxy* groups has been observed previously [4].

The experiments described here suggest that malonyl derivatives require some care in handling. NMR spectral measurements of newly isolated glycosides should be carried out initially at room temperature and in the absence of D20, methanol-d4 or other solvents containing exchangeable deuterium. The appearance of an acetyl methyl signal as the compound is being heated is confirmation that the precursor is a malonyl derivative. If plant extracts are found that contain corresponding pairs of acetates and malonates it is conceivable that in some instances the acetates will have arisen as artifacts during the work-up procedures.

*The exchange has been observed earlier in pelargonidin 3-malonylsophoroside dissolved in D20/DCI [2] and in luteolin 7-*O-(6"-O-matonyl)-fl-D-glucopyranoside* dissolved in pyridine-d5/D20 [3].

## EXPERIMENTAL

NMR spectra were determined at 60, 100 or 270MHz. FABMS were determined in the positive ion mode using a glycerol matrix and xenon as the fast atom beam {20 mA at 7 or 8 kV). Compounds 1-3 were isolated earlier from flowers of *Gerberajamesonii* [5]. The potassium salt of 2 was isolated from flowers of Heavenly Blue morning glory *(lpomoea tricolor) (S.* Asen, unpublished data). FABMS: 589 [M + K] ~, 503 [M + K -malonyl] +, 303 [quercetin + H] ~. 6,8-Dideuteriokaempferol 3-3-D-glucoside 6"-trideuterioacetate was isolated from the exchange/decarboxylation reaction by evaporating the solvent in a stream of N,. FABMS: 496 [M + HI ~ 289 [dideuteriokaempferol +]-I]

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