JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 577–581.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.586

Research Article

Synthesis of (±)-6,6-[$^2H_6$]dimethyl-11-nor-Δ^9-tetrahydrocannabivarin-9-carboxylic acid

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Summary

Starting from divarinol (**4**) using previously published procedures, (±)-6,6-[$^2H_6$]Dimethyl-11-nor-Δ^9THCV-9-carboxylic acid (**3**) was synthesized for use as an internal standard in GC/MS analysis of 11-nor-Δ^9THCV-9-carboxylic acid (**2**). The detection of **2** distinguishes the use of marijuana from the ingestion of Marinol®. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: (±)-6,6-[$^2H_6$]dimethyl-11-nor-(Δ^9-THCV-9-carboxylic acid; 11-nor-Δ^9-THCV-9-carboxylic acid; Δ^9-THCV; marijuana; Marinol®

In the past several years, forensic toxicologists have been searching for a scientifically acceptable way to distinguish the ingestion of Marinol®, a prescription drug that contains synthetic Δ^9-tetrahydrocannabinol (Δ^9-THC), from the use of marijuana, an illegal drug. We have previously proposed[1] that Δ^9-tetrahydrocannabivarin (Δ^9-THCV, **1**), a C3 homolog of Δ^9-THC and a natural component of cannabis plant, could be used as a marker for the ingestion of marijuana (or a related product) versus Marinol® because **1** does not exist in Marinol®. Recently, we reported that 11-nor-Δ^9-tetrahydrocannabivarin-9-carboxylic acid (**2**) is the major urinary metabolite of **1** through both *in vitro* metabolism and clinical studies.[2,3] It was concluded from these studies that the presence

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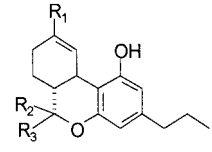
Received 12 December 2001
Accepted 27 February 2002

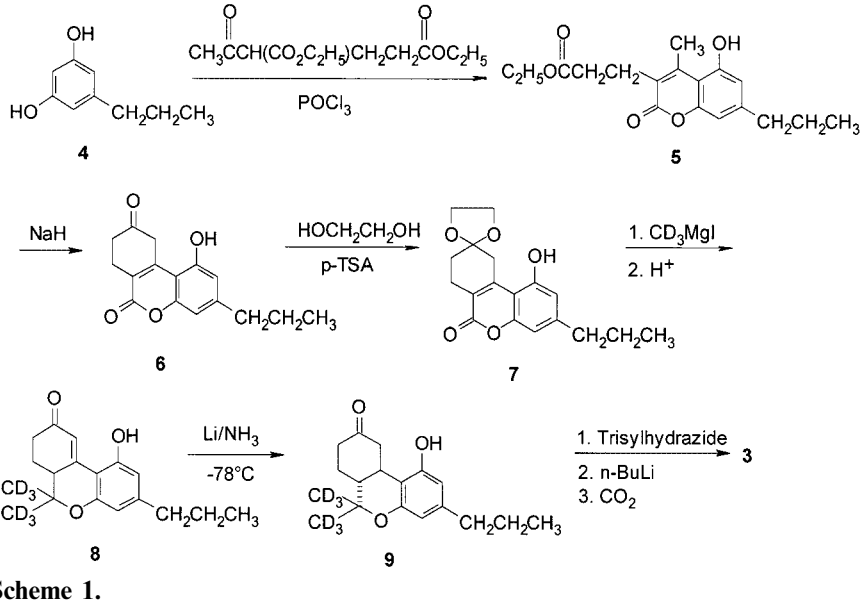
of **2** in a urine specimen would confirm that the subject must have used marijuana or a related product. The deuterium labeled analog, **3**, was required for quantitative analysis by GC/MS. Because of this important forensic application, in this communication, we wish to report the synthesis of **3** starting from divarinol (**4**) using our previously published procedure[4] for the synthesis of d_6-11-nor-9-carboxy-Δ^9-THC, the C5 analog. Although the latter could also be used as an internal standard, **3** is superior in terms of analytical performance such as dynamic linearity range of analysis (1–1000 ng/ml versus 2–50 ng/ml for urine specimens) (see Figure 1).

As shown in Scheme 1, treatment of **4** with diethyl 2-acetylglutarate and $POCl_3$ gave the bicyclic coumarin **5** in 61% yield in several crops as



1 $R_1 = R_2 = R_3 = CH_3$
2 $R_1 = CO_2H$, $R_2 = R_3 = CH_3$
3 $R_1 = CO_2H$, $R_2 = R_3 = CD_3$

Figure 1.



Scheme 1.

a white solid: m.p. 161–163°C; EI-MS *m*/*z*: 318 (M^+, 14%), 244 (100%); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 6.64 (d, $J = 1.1$ Hz, 1 H), 6.46 (m, 2 H including one exchangeable), 4.15 (q, $J = 7.2$ Hz, 2 H), 2.99 (t, $J = 7.5$ Hz, 2 H), 2.66 (s, 3 H), 2.54 (m, 4 H), 1.61 (m, 2 H), 1.27 (t, $J = 7.1$ Hz, 3 H), 0.93 (t, $J = 7.3$ Hz, 3 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 173.5, 162.1, 154.7, 153.5, 150.4, 146.7, 121.3, 111.9, 108.6, 107.8, 60.8, 37.6, 32.9, 23.7, 22.8, 19.2, 14.1, 13.7. *Anal*. Calculated for $C_{18}H_{22}O_5$: C, 67.92; H, 6.92. Found: C, 67.82; H, 7.33.

Cyclization of **5** by treatment with NaH in dry DMSO gave **6** in 52% as a white crystalline solid: m.p. 238.5–240°C; EI-MS *m*/*z* (as TMS derivative): 344 (M^+,100%); ^{1}H-NMR ($CDCl_3$) δ: 7.54 (s,1 H, exchangeable), 6.74 (br s, 1 H), 6.57 (br s, 1 H), 4.20 (s, 2 H), 3.08 (t, $J = 7.1$ Hz, 2 H), 2.70 (t, $J = 7.1$ Hz, 2 H), 2.58 (t, $J = 7.7$ Hz, 2 H), 1.65 (m, 2 H), 0.95 (t, $J = 7.4$ Hz, 3 H); ^{13}C-NMR (d_6-DMSO, 75 MHz) δ: 207.7, 166.9, 160.1, 156.1, 155.9, 153.9, 146.6, 118.8, 106.0, 105.9, 43.3, 37.1, 36.9, 23.5, 23.0, 13.7. *Anal*. Calculated for $C_{16}H_{16}O_4$: C, 70.59; H, 5.88. Found: C, 70.30; H, 5.97.

By treatment of **6** with ethylene glycol and *p*-toluenesulfonic acid in benzene, the ketal **7** was obtained as a pale yellow solid (m.p. 157–159°C) in 90% yield and was identified by GC/MS: *m*/*z* (calculated MW 316 for $C_{18}H_{20}O_5$): 316 (M^+,100%). Without further purification, **7** was treated with commercially available CD_3MgI (Aldrich Chemical Company, 99+ atom% D) followed by acidic hydrolysis to afford the racemic α,β-unsaturated ketone **8** in 60% yield as a pale-yellow crystalline solid: m.p. 220–223°C; EI-MS *m*/*z*: 292 (M^+,65%), 274 (100%); ^{1}H-NMR ($CDCl_3$) δ: 10.7 (s, 1 H, exchangeable), 8.04 (d, $J = 2.1$ Hz, 1 H), 6.52 (d, $J = 1.5$ Hz, 1 H), 6.24 (d, $J = 1.5$ Hz, 1 H), 2.82 (m, 1 H), 2.66 (m, 1 H), 2.44–2.57 (m, 3 H), 2.19 (m, 1 H), 1.56–1.79 (m, 3 H), 0.93 (t, $J = 7.3$ Hz, 3 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 204.0, 159.2, 156.4, 154.1, 149.3, 122.2, 109.4, 108.9, 105.9, 77.1, 44.7, 37.9, 36.4, 24.3, 23.8, 13.9. *Anal*. Calculated for $C_{18}H_{16}D_6O_3$: C, 73.89; H, 7.81. Found: C, 73.45; H, 7.62.

Birch reduction of **8** gave *trans*-ketone **9** as a white solid in 60% yield: m.p. 171–172°C; EI-MS *m*/*z*: 294 (M^+, 100%); ^{1}H-NMR ($CDCl_3$) δ: 8.17 (s, 1 H, exchangeable), 6.32 (d, $J = 1.5$ Hz, 1 H), 6.23 (d, $J = 1.5$ Hz, 1 H), 4.20 (ddd, $J = 2.4$, 3.0, and 15.1 Hz, 1 H), 2.89 (m, 1 H), 2.63 (m, 1 H), 2.51 (m, 1 H), 2.43 (t, $J = 7.2$ Hz, 2 H), 2.14 (m, 2 H), 1.97 (ddd, $J = 2.6$, 12.1, and 14.2 Hz, 1 H), 1.52–1.63 (m, 3 H), 0.92 (t, $J = 7.3$ Hz, 3 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 215.6, 155.5, 154.4, 143.3, 108.9, 107.8 (C-2 and C-4), 76.3, 47.2, 44.7, 40.8, 37.6, 34.7, 26.9, 24.1, 13.9.

Anal. Calculated for $C_{18}H_{18}D_6O_3$: C, 73.45; H, 8.46. Found: C, 73.77; H, 8.20.

Finally, **9** was converted to **3** by a Shapiro reaction as reported previously[4] in 54% yield as a crude product which contained ca. 84% of the desired Δ^9-isomer. Recrystallization twice from ether–hexane gave pure **3** as a white solid (99% by HPLC, $t_r = 4.48$ min using a Microsorb® C18 column, 3.9×100 mm, CH_3CN–H_2O–HOAc 60:40: 0.05 at 1.0 ml/min, UV detector at 228 nm). The ^{1}H-NMR spectrum was identical to the previously reported spectrum of **2**,[2] the unlabeled analog of **3**, except for the total absence of the signals for the *gem*-dimethyl protons at the C-6 position. Specifically, the two singlets at δ 1.44 (3 H)

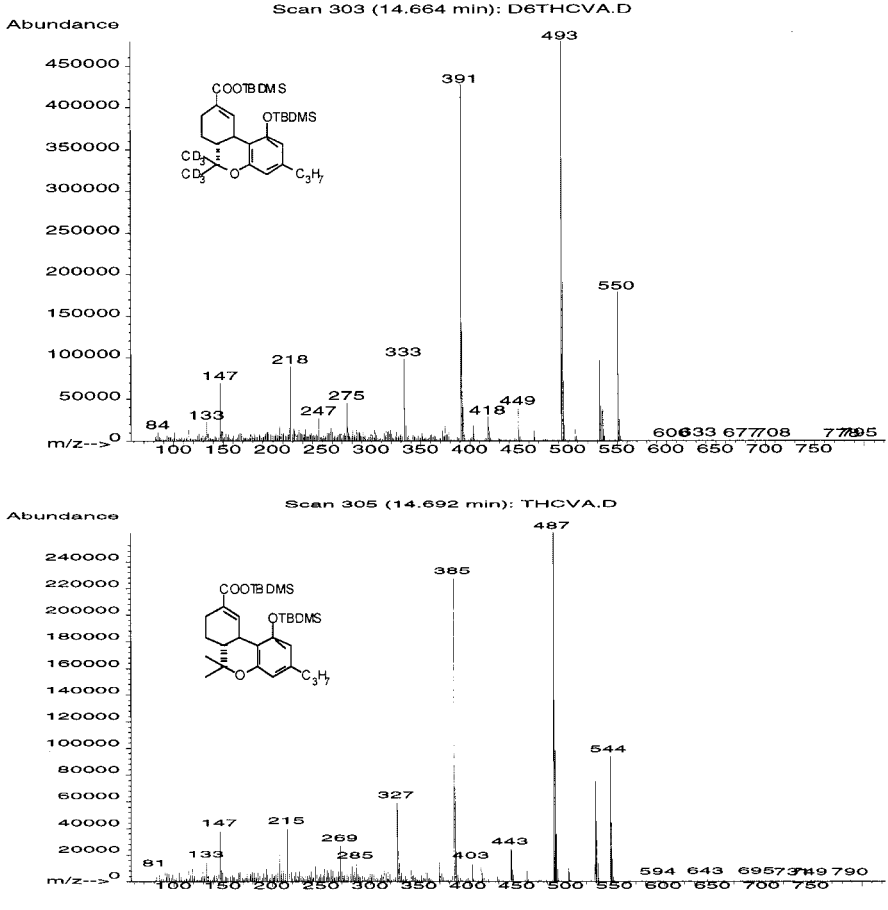


Figure 2. Comparison of EI-MS spectra of 3 (top) and 2 (bottom) as TBDMS derivatives

and 1.12 (3 H) disappeared, indicating full deuteration of these methyl groups. The structure of **3** was further confirmed by comparing the EI-MS spectra of TBDMS derivatives of **3** ($M^+ = 550$) and **2** ($M^+ = 544$) obtained by heating with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide containing 1% TBDMSCI (Figure 2). Our published data show[3] that **3** is a superior internal standard to its C5 analog in GC-MS analysis of **2**.

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