Indian Journal of Chemistry
Vol. 42B, May 2003, pp. 1190-1192

Note

Revised structure for spatozoate, a metabolite of *Spatoglossum variabile*

Kur-Ta Cheng [a], Dodda Rajasekhar [b], Sheng-Tung Huang [a], Feng-Lin Hsu [b,*] & Gottumukkala V Subbaraju [c,*]

[a]Department of Biochemistry, Taipei Medical University, Taipei 110, Taiwan R.O.C.
[b]School of Pharmacy, Taipei Medical University, Taipei 110, Taiwan R.O.C.
and
[c]Laila Impex R&D center, Unit I, Phase III, Jawahar Autonagar, Vijayawada 520 007, India

Received 13 December 2001; accepted (revised) 14 August 2002

Structure of spatozoate, *n*-butyl 2-benzoyloxymethylbenzoate **1**, a metabolite of *Spatoglossum variabile* has been revised into benzyl *n*-butyl phthalate **2** by synthesis. NMR spectral data of synthetic **2** agrees well with those reported for the natural spatozoate.

Spatozoate **1** was isolated as a new metabolite from the brown alga *Spatoglossum variabile*[1] and its structure was derived as *n*-butyl 2-benzoyloxymethylbenzoate based on spectroscopic evidence. A careful scrutiny of the reported proton NMR data for spatozoate, however, revealed that the assignment of close chemical shifts (δ 7.70 for H-3 and 7.74 for H-6) for aromatic protons adjacent to oxymethylene and carboxyl groups, respectively, appears rather untenable. To ascertain the validity of the structure assigned for spatozoate, we have synthesized **1** in a straight forward synthetic route starting from **3** (**Scheme I**). As expected, the NMR data of **1** did not corroborate with the data reported for spatozoate and revealed the necessity of structure revision. Further examination of NMR spectral data of spatozoate led to the proposal of an alternative isomeric structure **2**, possible for spatozoate. We have prepared **2** by a simple procedure starting from phthalic anhydride (**Scheme II**) and found that the data reported for natural metabolite agree well with those of **2** confirming that the structure of spatozoate is indeed **2**. The details of synthesis of **1** and **2** are presented below.

n-Butyl 2-benzoyloxymethylbenzoate **1** was prepared from 2-hydroxymethylbenzoic acid **3** in two steps (**Scheme I**). The desired **3** was obtained, in turn, by the reduction of phthalimide using Zn-NaOH in presence of copper sulphate in 66% yield[2]. **3** was treated with *n*-butyl bromide and potassium carbonate to give *n*-butyl 2-hydroxymethylbenzoate **4** in 13% yield. **4** was converted into **1** by treating it with benzoyl chloride and triethylamine in 58 % yield. The data of synthetic **1** (**Table I**) have been found to be inconsistent with those reported for spatozoate, particularly for the protons H-3 to H-6.

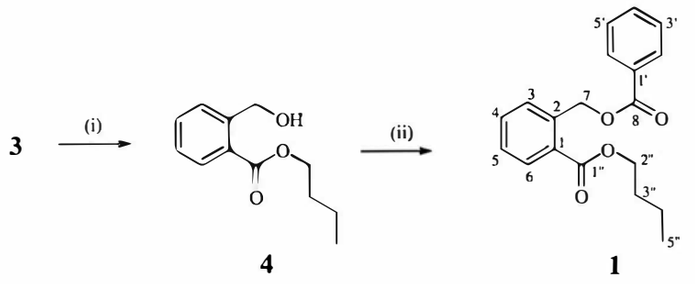
The close chemical shifts for H-3 and H-6; H-4 and H-5 points out that the values may fit better for phthalate unit[3]. Based on this observation, we have proposed an alternative isomeric structure, **2**, for spatozoate. We have prepared **2** by the reaction of phthalic anhydride with *n*-butyl alcohol to give mono *n*-butyl phthalate **6** in about 60% yield. **6** was smoothly converted into **2** (**Scheme II**) by treating it with benzyl bromide in presence of potassium carbonate in 88% yield. The spectral data of **2** were in good agreement with those reported for the natural metabolite confirming the revised structure **2** for spatozoate.

It is interesting to note that several phthalate esters were reported to possess cytotoxic[4] and insect repellant[5] activities. Literature survey on **2** revealed that it is cytotoxic to human fibroblast and melanoma cells (SK-Mel/27) in culture[4].

Experimental Section

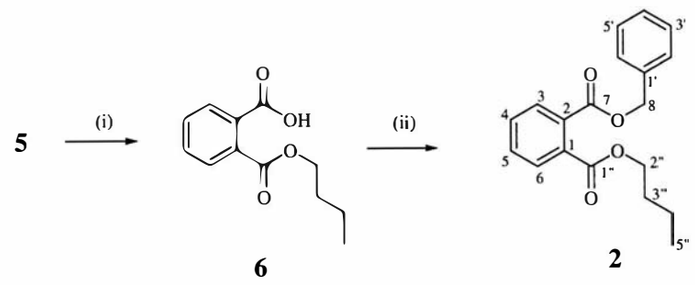
Melting points were measured on a BUCHI-540 melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer RX I FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX 500 NMR spectrometer and EI MS on a Hewlett Packard 5989 B mass spectrometer. Mixtures of hexane and ethyl acetate were used for elution in the chromatographic purification of liquid compounds.

2-Hydroxymethylbenzoic acid 3. To a mixture of zinc powder (9.0 g, 0.137 moles) and crystallised copper(II) sulphate (50 mg) in 2 mL water at 5°C was added 16.5 mL of aq. NaOH (20%) followed by phthalimide (7.35 g, 0.05 moles) in four portions over a period of 30 min and stirring was continued for further 30 min. After this period, water (20 mL) was added and the contents were heated on a water bath for 3 hr. The reaction mixture was cooled and filtered



(i) *n*-BuBr, K_2CO_3, Acetone; (ii) BzCl, TEA, CH_2Cl_2

Scheme I



(i) *n*-BuOH, H_2SO_4; (ii) BzBr, K_2CO_3, Acetone

Scheme II

to remove zinc and added excess conc. HCl. The precipitated solid was filtered and washed with water (3×10 mL), dried to give **3** (5.0 g, 65.8%), m.p. 127-28°C; IR (KBr): 2654-2925 br (hydroxyl and carboxylic acid) 1683 (carboxyl carbonyl) and 1274 cm^{-1}; ^1H NMR (500 MHz, d_4-MeOH): δ 7.96 (1H, brd, J=7.5 Hz, H-6), 7.64 (1H, brd, J=7.7 Hz, H-3), 7.55 (1H, brt, J = 7.6 Hz, H-4), 7.35 (1H, brt, J=7.6 Hz, H-5), 4.92 (2H, s, H-7), 4.85 (brs, -OH).

***n*-Butyl 2-hydoxymethylbenzoate 4.** A mixture of 2-hydroxymethylbenzoic acid **3** (0.76 g, 5.0 mmoles), *n*-butyl bromide (0.82 g, 6.0 mmoles), potassium carbonate (0.75 g) and dry acetone (15 mL) was refluxed for 10 hr. After this period, K_2CO_3 was filtered off, the solvent removed and the residue was purified over silica gel to give **4** (0.13 g, 12.5%) as colourless liquid; IR (Neat): 3608, 1747 and 1287 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) : δ 7.99 (1H, brd, J=8.3 Hz, H-6), 7.51 (1H, td, J=7.5, 0.8 Hz, H-4), 7.43 (1H, brd, J=7.4 Hz, H-3), 7.35 (1H, td, J=8.2, 0.5 Hz, H-5), 4.75 (2H, s, H-7), 4.32 (2H, t, J=6.6 Hz, H-2″), 1.75 (2H, m, H-3″), 1.47 (2H, m, H-4″), 0.97 (3H, t, J=7.4 Hz, H-5″).

***n*-Butyl 2-benzoyloxymethylbenzoate 1.** To a mixture of *n*-butyl 2-hydroxymethylbenzoate **4** (104 mg, 0.5 mmoles), triethylamine (86 mg) and dichloromethane (10 mL) at 0 °C was added benzoyl chloride (120 mg, 0.85 mmoles) over a period of 15 min. The contents were allowed to warm-up to room temperature and stirring was continued for further 4 hr. The reaction mixture was diluted with dichloromethane (30 mL) and washed with saturated sodium bicarbonate solution followed by water and the organic layer was dried over anhydrous sodium sulphate. The residue obtained after removal of the solvent was purified over silica gel to give *n*-butyl 2-benzoyloxymethylbenzoate **1** (90 mg, 57.7%); IR (Neat): 1720 and 1269 cm^{-1}; EIMS : m/z 207 [M^+-benzoyl, 72], 133 (100), 105 [$PhCO^+$, 71]; ^1H and ^{13}C NMR : see **Table I**.

Mono *n*-butyl phthalate 6. To a solution of phthalic anhydride **5** (500 mg, 3.38 mmoles) in *n*-butyl alcohol (4 mL) was added two drops of conc. H_2SO_4 and refluxed for 15 min. After this period, *n*-butyl alcohol was evaporated under reduced pressure and ethyl acetate (20 mL) was added and washed with water, dried over anhydrous sodium sulphate and solvent evaporated to give a syrupy liquid, which was purified by chromatography over silica gel column to give mono *n*-butyl phthalate **6** (446 mg, 59.6%) as

Table I — ^1H and ^{13}C NMR data of spatozoate, 1 and 2

Position	Spatozoate[a,b]		Synthetic **1**[c]		Synthetic **2**[c]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		132.6		129.2		132.5
2		135.0		137.6		135.5[e]
3	7.70, *m*	130.8	7.49-7.57, *m*[d]	127.7	7.70, *m*	130.9
4	7.51, *td* (7.5,1.5)	128.3	7.49-7.57, *m*	133.0	7.50-7.52, *m*	128.8
5	7.53, *td* (7.5,1.5)	129.1	7.38, *brt* (7.5)	128.1	7.50-7.52, *m*	129.0
6	7.74, *dd* (7.5, 1.5)	131.1	8.00, *dd* (7.6, 0.9)	130.8	7.73, *m*	131.1
7	5.32, *s*	67.5	5.76, *s*	65.0		167.6
8		167.6		166.1	5.33, *s*	67.4
1'		131.9		130.1		131.8[e]
2', 6'	7.47, *dd* (8.3,1.8)	128.6	8.08, *dd* (8.1,1.0)	129.6	7.40, *brd* (7.3)	128.6
3', 5	7.30, *td* (8.3,1.8)	128.4	7.43, *brt* (7.8)	128.3	7.30-7.37, *m*	128.4
4'	7.12, *dd* (8.5, 1.8)	128.0	7.49-7.57, *m*	132.2	7.30-7.37, *m*	128.3
1"		167.4		166.9		167.4
2"	4.23, *t* (7.0)	65.6	4.29, *t* (6.7)	65.0	4.18, t (6.8)	65.5
3"	1.75, *m*	30.5	1.72, *m*	30.6	1.62, *m*	30.5
4"	1.42, *m*	19.2	1.44, *m*	19.2	1.37, *m*	19.1
5"	0.90, *t* (7.4)	13.7	0.93, *t* (7.5)	13.6	0.91, *t* (7.4)	13.7

[a]Data taken from reference 1; [b] ^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz, $CDCl_3$; [c] ^1H NMR: 500 MHz, ^{13}C NMR: 125 MHz, $CDCl_3$; [d]Underlined chemical shifts showed large variation in relation to the data reported on natural spatozoate, ref. 1; [e]Assignments may be interchanged.

colourless liquid; IR (Neat): 3078, 2960, 1724 and 1288 cm^{-1}; ^1H NMR (500, $CDCl_3$) : δ 7.84 (1H, brd, *J*=7.9 Hz, H-3); 7.66 (1H, brd, *J*=7.2 Hz, H-6); 7.54 (1H, td, *J*=7.3, 1.1 Hz, H-5); 7.51 (1H, td, *J*=7.4, 1.1 Hz, H-4), 4.29 (2H, t, *J*=6.7 Hz, H-2″), 1.69 (2H, m, H-3″), 1.40 (2H, m, H-4″), 0.90 (3H, t, *J*=7.4 Hz, H-5″).

Benzyl *n*-butyl phthalate 2. A mixture of **6** (222 mg, 1 mmole), benzyl bromide (330 mg, 1.9 mmoles), potassium carbonate (160 mg) and acetone (5 mL) was refluxed for 3 hr. The usual work-up afforded a residue, purified over silica gel to yield **2** (275 mg, 88%) as colourless liquid; IR (Neat) :1726 and 1285 cm^{-1}; EIMS: m/z 312 [M^+, 4], 238 (5), 206 (41), 150 (16), 149 (100), 105 (17), 104 (20); ^1H and ^{13}C NMR : see **Table I.**

Acknowledgement

The authors thank the National Science Council, R.O.C. (Grant No. NSC89-2317-B-038 -002) for the financial support to DR and the Instrument Centre, Taipei Medical University for spectral data.

References

1 Atta-ur-Rahman, Choudhary M I, Hayat S, Khan A M, Ahmad A & Malik S, *Phytochemistry*, 52, **1999**, 495.

2 Furniss B S, Hannaford A J, Smith P W G & Tatchell A R, In: *Vogel's textbook of practical organic chemistry* (John Wiley & Sons, New York), **1989**, p. 1066.

3 Rao G N, Kumar P M, Dhandapani V S, Krishna T R & Hayashi T, *Fitoterapia*, 71, **2000**, 82.

4 Babich H & Borenfreund E, *J Pharm Sci*, 79, **1990**, 592.

5 *The Merck Index: An encyclopedia of chemicals, drugs and biologicals*, (Merck & Co., Whitehouse station, New Jersey), 12th ed., **1996**, p. 550.