Indian Journal of Chemistry
Vol. 47B, December 2008, pp. 1865-1870

Radermachol and naphthoquinone derivatives from *Tecomella undulata*: Complete ^{1}H and ^{13}C NMR assignments of radermachol with the aid of computational ^{13}C shift prediction

Pahup Singh*[a], Poonam Khandelwal[a], Noriyuki Hara[b], Teigo Asai[b] & Yoshinori Fujimoto[b]

[a] Centre of Advanced Studies, Department of Chemistry, University of Rajasthan, Jaipur 302 004, India
[b]Department of Chemistry and Materials Science, Tokyo Institute of Technology, O-okayama,
Meguro-ku, Tokyo 152-8551, Japan
E-mail: pahupsingh@yahoo.co.uk

Received 6 June 2008; accepted (revised) 4 September 2008

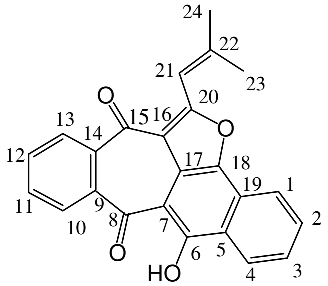
Petroleum ether extract of the heartwood of *Tecomella undulata* affords radermachol, an unusual rare pigment and 2-isopropenylnaphtho[2,3-*b*]furan-4,9-quinone along with lapachol, tecomaquinone-I, dehydro-α-lapachone, α-lapachone, β-lapachone, cluytyl ferulate, stigmasterol and β-sitosterol. Radermachol and 2-isopropenylnaphtho [2,3-*b*]furan-4,9-quinone are being reported for the first time from genus *Tecomella*. Complete assignments of ^{1}H and ^{13}C NMR signals of polyketide, radermachol **1**, have been achieved by the ^{13}C NMR chemical shift prediction using *ab initio* MO and DFT/GIAO methods in addition to 2D-NMR techniques.

Keywords: Radermachol, 2-isopropenylnaphtho[2,3-*b*]furan-4,9-quinone, chemical shift prediction, *Tecomella undulata*, computational chemistry

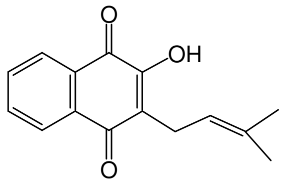
Tecomella undulata (G. DON) Seem (syn: *Tecoma undulata*) Bignoniaceae is a small attractive tree when in full bloom; flourishes in dry districts and also at other places where irrigation is not possible. In indigenous system of medicine, it is described to be useful[1] in curing urinary discharges, enlargement of spleen, gonorrhoea, leucoderma and liver diseases. A perusal of literature reveals that quinonoid constituents have been reported from the heartwood and roots of this plant[2-8]. Its heartwood is a rich source of lapachol and appearance of further minor coloured bands in the PTLC prompted its reinvestigation in search of some more interesting pigments from this plant.

Radermachol **1** was isolated by Joshi *et al.* in 1984 from the roots of *Radermachera xylocarpa* K. Schum (Bignoniaceae), and reported to have antiinflammatory activity[9]. The unique pentacyclic aromatic structure of **1** was elucidated mainly by crystallographic study[9]. ^{13}C NMR signals, most of which are aromatic carbons, have remained unassigned. Total synthesis of **1** has been achieved by the same group[10]. A biosynthetic route of this compound, featuring dimerization of lapachol or the related furanonaphthoquinone, has been proposed[11]. There has been no record of detailed ^{1}H, ^{13}C and 2D NMR spectra of this highly aromatic compound **1**, since its first isolation. In pursuing the current interest[12] in the quinone constituents of the Bignoniaceae, herein is reported the isolation of radermachol **1** and 2-isopropenylnaphtho[2,3-*b*]furan-4,9-quinone **4** along with previously reported lapachol **2**, tecomaquinone-I **3**, dehydro-α-lapachone **5**, cluytyl ferulate **6,** α-lapachone **7**, β-lapachone **8**, stigmasterol and β-sitosterol from the heartwood of *Tecomella undulata*. The availability of **1** prompted the study on complete assignments of its ^{1}H and ^{13}C NMR signals.

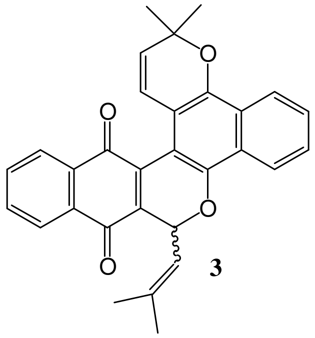
Radermachol isolated from *T. undulata* was identified by comparison of the NMR data with those of the reported values in addition to the resemblance of the other spectral (UV-Vis and MS) properties[9]. The ^{1}H and ^{13}C NMR data are listed in **Table I**. The heteronuclear multiple quantum coherence (HMQC) spectrum afforded information about the direct linkage of all protons with carbons. The heteronuclear multiple bond connectivity (HMBC) spectrum ($J_{\text{C–H}}$=10 Hz) showed correlations summarized in **Table I**, including those from the strongly chelated phenolic proton at δ 15.30 to C-5, C-6 and C-7. These data allowed the correct assigning of most of the signals. However, the two carbonyl carbons (C-8 and C-15) could not be connected to the right half of the molecule unambiguously, since there is no hydrogen atom within three-bond lengths from the carbonyl



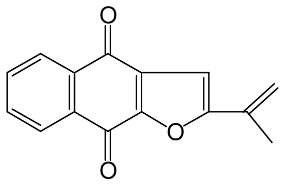
1



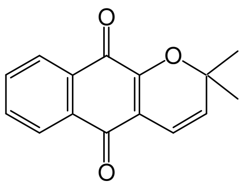
2



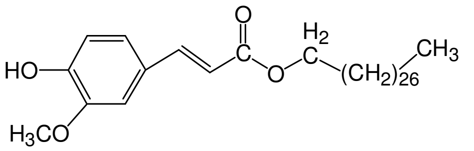
3



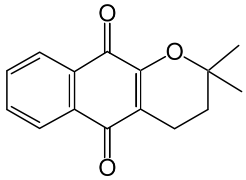
4



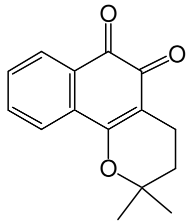
5



6



7



8

carbons (C-6, C-7, C16 and C-20 are all quaternary carbons). Thus, C-8/C-9/C-10/C-11 and C-15/C-14/C-13/C-12 were assigned as interchangeable pairs, although there is considerable difference between the chemical shifts (Δδ *ca.* 7 ppm) of the two carbonyl carbons. In addition, the two quaternary carbons, C-16 and C-17 (δ 117.52 and 117.78), were assigned also as an interchangeable pair. The expected three-bond HMBC correlation from H-21 to C-16 was not observed probably due to a small $J_{\text{C–H}}$ value. The three-bond correlation could not be detected, even when the spectra were recorded at the parameter of $J_{\text{C-H}}$=6.0 Hz. In an attempt to assign H-13 by a possible Nuclear Overhauser Effect (NOE) correlation from the isobutenyl proton signals, gradient enhanced NOE (GOESY) experiments[13,14] were carried out. Irradiation of (*Z*)-methyl (H_3-23) at δ 2.44 caused an NOE enhancement of H-1 (δ 8.17) in addition to H_3-24 (δ 2.17), but not to H-13. The result indicates that the isobutenyl group lies exclusively *syn* to the furan oxygen atom, which is consistent with that observed in the crystal structure[9].

Since it seemed difficult to obtain further information by 2D-NMR experiments, it was decided to apply the computational techniques for geometry optimization and ^{13}C chemical shift prediction. In Gauge-Independent Atomic Orbital (GIAO) method[15], the magnetic shielding tensors are calculated as the perturbation to the electronic structure in the ground state. This method is reported to be more preferred for the calculation of delocalized systems, for instance, aromatic compounds[16]. Furthermore, the level of the calculation at the stage of geometry optimization is known to be of importance in the results of magnetic shielding constants.

In the energy-minimized structure at the level of B3LYP/6-31G(d,p), the calculated bond distances (**Table II**) were in good accordance with that in the X-ray structure, and the boat conformation of the seven membered-ring was well reproduced; *i.e.* the four carbons (C-9, C-14, C-16 and C-17) forms the basal plane (dihedral angle = 4.0°), C-7 and C-8 are placed at the stern position, and C-15 at the prow. On the other hand, the calculated hydrogen bond distance

Table I — NMR data and HMBC correlations for radermachol **1**

Carbon No.	δ_H	δ_C (obs)	δc (calcd))	HMBC correlations
1	8.17 (d, 8.2)	119.61 (d)	117.71	C-3, C-5, C-18
2	7.78 (td, 8.5, 1.1)	130.92 (d)	128.57	C-4, C-19
3	7.58 (td, 8.3, 1.1)	125.54 (d)	121.28	C-1, C-5
4	8.61 (d, 8.4)	125.92 (d)	126.02	C-19, C-6
5	-	123.45 (s)	121.60	
6	-	164.14 (s)	164.67	
7	-	107.76 (s)	108.30	
8	-	192.61(s)	188.52	
9	-	135.90 (s)	134.12	
10	8.57 (m)	132.54 (d)	130.91	C-8, C-12, C-14
11	7.79 (m)	131.98 (d)	128.50	C-9, C-10, C-13, C-14
12	7.79 (m)	133.32 (d)	129.56	
13	8.39 (m)	131.51 (d)	130.55	C-9, C-11
14	-	138.58 (s)	137.21	
15	-	185.73 (s)	181.50	
16	-	117.52 (s)	118.14	
17	-	117.78 (s)	118.68	
18	-	141.55 (s)	138.09	
19	-	124.87 (s)	122.73	
20	-	160.83 (s)	159.21	
21	7.24 (br sept, 1.3)	114.22 (d)	115.32	C-20, C-23, C-24
22	-	148.30 (s)	148.45	
23.	2.44 (s)	21.89 (q)	23.09	C-20, C-21, C-22, C-24
24	2.17 (brs)	28.64 (q)	30.06	C-21, C-22, C-23
OH	15.30 (s)			C-5, C-6, C-7

Spectra were recorded at 500 MHz for δ_H and 125 MHz for δ_C in $CDCl_3$.

of 1.584 Å and the angle O-H-O of 148.3° were slightly different from the X-ray data (1.50 Å and 153°, respectively).

The GIAO calculation at the mPW1PW91/6-31G(d,p) level[17] provided the isotropic magnetic shielding constants, which were expressed as the chemical shifts from TMS. The resulting δ values, along with the observed data, are included in **Table I**. The predicted chemical shifts for the C-8 and C-15 carbonyl carbons are δ 188.52 and 181.50, respectively. Hence, the C-8 and C-15 resonances of **1** were assigned as δ 192.61 and 185.73, respectively. Accordingly, the carbon signals for the left-handed benzene ring, *i.e.*, C-9 to C-14, were assigned as listed in **Table I**. The signals of C-16 and C-17 were calculated to be δ 118.14 and 118.68, respectively. The signals, δ 117.52 and 117.78, were tentatively assigned to C-16 and C-17, respectively (the errors being +0.62 and +0.90 ppm), although the difference is within the experimental error (*vide infra*). The predicted chemical shifts for the other carbons were in good agreement within the maximum experimental error of 4.26 (C-3) and the average deviation |Δδ| of 1.89 ppm. The difference between the calculated and the observed ^{13}C chemical shifts are depicted in **Figure 1**.

In conclusion, the complete ^1H and ^{13}C NMR assignments of radermachol **1** was achieved by 2D NMR techniques combined with the chemical shift prediction by calculation at B3LYP/6-31G(d,p) minima using DFT/GIAO. The computational method has been applied for the structure elucidation and revision of natural products[18,19].

Experimental Section

^1H and ^{13}C NMR experiments were carried out in a $CDCl_3$ solution (7.0 mg/mL) at 30°C on a Bruker Avance DRX500 spectrometer equipped with a 5 mm multinuclear inverse probehead working at 500.130 MHz for ^1H and 125.758 MHz for ^{13}C nuclei. Chemical shifts were referenced to the internal TMS signal (at δ 0.00) for both ^1H and ^{13}C nuclei.

Table II — Difference between observed and calculated bond lengths in **1**

Bond	Bond length (Å)		Difference
	Observed[a]	Calculated	
C1-C2	1.370	1.381	-0.011
C2-C3	1.396	1.412	-0.016
C3-C4	1.368	1.381	-0.013
C4-C5	1.411	1.414	-0.003
C5-C19	1.426	1.428	-0.002
C19-C1	1.408	1.414	-0.006
C5-C6	1.426	1.444	-0.018
C6-O2	1.343	1.327	0.016
C6-C7	1.406	1.420	-0.014
C7-C8	1.435	1.454	-0.019
C8-O3	1.253	1.258	-0.005
C8-C9	1.497	1.508	-0.011
C9-C10	1.399	1.411	-0.012
C10-C11	1.370	1.387	-0.017
C11-C12	1.376	1.394	-0.018
C12-C13	1.377	1.387	-0.010
C13-C14	1.395	1.408	-0.013
C14-C9	1.409	1.421	-0.012
C14-C15	1.507	1.513	-0.006
C15-O4	1.226	1.233	-0.007
C15-C16	1.455	1.461	-0.006
C16-C17	(b)	1.436	
C17-C7	1.430	1.431	-0.001
C17-C18	1.365	1.383	-0.018
C18-O1	1.382	1.370	0.012
C18-C19	1.406	1.413	-0.007
C16-C20	1.382	1.401	-0.019
C20-O1	1.377	1.364	0.013
C20-C21	1.432	1.439	-0.007
C21-C22	1.345	1.356	-0.011
C22-C23	1.503	1.507	-0.004
C22-C24	1.493	1.506	-0.013

(a) Adopted from reference 1 (b) Not reported

Plant Material and Extraction: The heartwood of *Tecomella undulata* was collected from the Jodhpur district of Rajasthan. Completely air-dried and finely powdered heartwood shavings (3 kg) were extracted with petroleum ether (60-80^o) over a water-bath for 3×12 hr. Extract was concentrated to dryness *in vacuo*, taken in Et_2O and then extracted with 2*N* Na_2CO_3 aqueous solution and the sodium carbonate soluble fraction was acidified with 2*N* HCl giving yellow mass (100 g) which was characterized as lapachol.

Lapachol, **2**: Bright yellow needles, yield 100 g, m.p. 139-40°C, (*lit.*[3] 140°C); R_f 0.65; UV-Vis (EtOH): 251, 278, 333 nm; IR (KBr): 3332, 1660, 1637, 1591 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.68 s and 1.78 s (2×=C-$(CH_3)_2$), 5.20 tq (*J*=7,1 Hz,=CH), 3.30 dbr (*J*=7 Hz,-CH_2-), 7.32 s (OH), 7.67 and 7.35 each dd (*J*=8,8 Hz, H-6 and H-7) and 8.07 and 8.12 dd each (*J*=8,2 Hz, H-5 and H-8); MS: *m/z* 242 [M$^+$] ($C_{15}H_{14}O_3$).

From Na_2CO_3 insoluble fraction (Neutral fraction) were afforded nine compounds by CC over Brockman alumina (deactivated with 10% aqueous glacial acetic acid) followed by preparative TLC over silica gel plates.

Radermachol, 1: Chromatographic separation of the neutral fraction over neutral alumina (eluent, benzene-ethyl acetate, 3:1) followed by reversed-phase HPLC purification (an ODS column, 15 cm × 5 mm i.d.; solvent, methanol-H_2O 10:1) afforded radermachol **1** as a red crystalline solid (70 mg), m.p. 212-13°C (from petroleum ether-benzene) (*lit.*[9] 217–18°C), R_f 0.80; UV-Vis ($CHCl_3$): 252, 276, 305, 330, 363 460 nm; IR (KBr): 1730, 1630, 1600, 1578 cm^{-1};

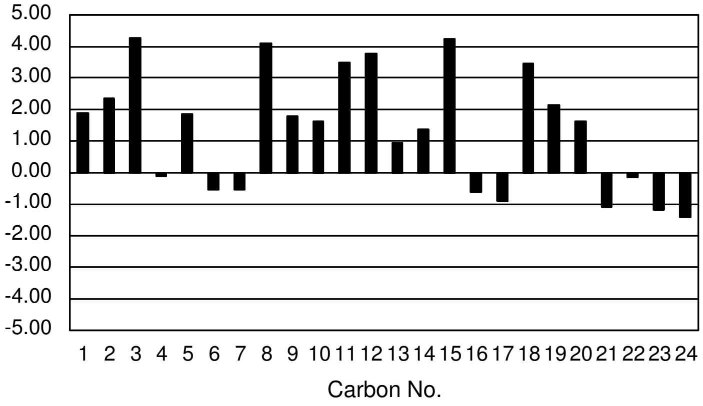


Figure 1 — Differences between observed and calculated ^{13}C NMR chemical shifts for radermachol **1**, $\Delta\delta_C$(obs)–δ_C (calcd) values are shown.

EIMS (70 eV): *m/z* (%) 368 ($[M]^+$, $C_{24}H_{16}O_4$, 63), 353 (100), 314 (11), 299 (8), 239 (10), 213 (8), 177 (15), 77 (7), 69 (7), 44 (6), 43 (6), 41(6).

Tecomaquinone-I, 3: Blue green crystals, yield 100 mg, m.p. 192-93°C (MeOH), (*lit.*[21] 198-99°C), R_f 0.70; UV-Vis (EtOH): 271, 343, 422 and 592 nm; IR (KBr): 1664 and 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.64 sbr ($-CH_3$) and 1.66 sbr ($-CH_3$), 1.60 d (*J* = 1.28 Hz, $=C–CH_3$), 2.05 d (*J*=1.33 Hz, $=C–CH_3$), 5.58 d (*J* = 9.65 Hz, 1H), 6.16 d (*J* = 9.65 Hz, 1H), 6.42 d (*J* = 9.34 Hz, 1H), 5.45 dm (*J* = 9.34, 1.33, 1.28 Hz, 1H), 7.51m (2 × Ar–H), 7.75 m (2×Ar–H), 8.17 m (4×Ar-H); MS: *m/z* 448 $[M^+]$($C_{30}H_{24}O_4$). On refluxing tecomaquinone-I with zinc dust gave tectol, m.p. 215-16°C.

2-Isopropenylnaphtho[2,3-*b*]furan-4,9-quinone, 4: Yellow crystals, yield 30 mg, m.p. 172-73°C, R_f 0.65; IR (KBr): 1660, 1665 cm^{-1}; MS: *m/z* 238 $[M^+]$ ($C_{15}H_{10}O_3$); ^1H NMR ($CDCl_3$): δ 2.14 s ($=C-CH_3$), 5.35 sbr and 5.96 sbr ($-C=CH_2$), 6.84 s (=CH), 7.74 m (2×Ar-H), 8.30 m (2×Ar-H). Above observations led to its identity as 2-isopropenylnaphtho [2,3-*b*]furan-4,9-quinone. This quinone was earlier isolated from the wood of *Radermachera sinica*[22].

Dehydro-α-lapachone, 5: Orange red needles, yield 150 mg, m.p. 142-43°C, R_f 0.60; UV-Vis (EtOH): 267, 276 (sharp), 333 and 434 nm; IR (KBr): 1680, 1640, 1632 and 1580 cm^{-1}; MS: *m/z* 240 $[M^+]$ ($C_{15}H_{12}O_3$); ^1H NMR ($CDCl_3$): δ 1.54 s (2×CH_3), 5.76 d (*J*=11Hz,=CH), 6.73 d (*J*=11Hz, =CH), 7.72 m (2×Ar-H), 8.13 m (2×Ar-H). It was characterized as dehydro-α-lapachone[23].

Cluytyl ferulate, 6: White crystals, yield 30 mg, m.p. 79-80°C, (*lit.*[24] 80-82°C), R_f 0.50; IR (Nujol): 3540, 1710, 1635, 1500, 1380, 1350 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 t (*J*=7 Hz, $-CH_3$), 1.25 sbr (52H), 3.93 s ($-OCH_3$), 5.84 sbr (OH), 6.29 d (*J*=16 Hz, =CH-), 7.06 d (*J*=16 Hz, =CH-), 6.91 d (*J*=8 Hz, 1× Ar-H), 7.04 d (*J*=2 Hz, 1×Ar-H), 7.07 dd (*J*=8,2 Hz, 1×Ar-H); MS: *m/z* 586 $[M^+]$ ($C_{35}H_{66}O_4$). On acetylation with acetic anhydride it gave octacosanyl acetoxy ferulate, m.p. 96-97°C.

α-Lapachone, 7: Yellow needles, yield 40 mg, m.p. 117-18°C (petroleum ether), (*lit.*[23] 119°C), R_f 0.45; UV-Vis (EtOH): 251, 282, 332, 375 nm; IR (Nujol): 1678, 1610, 1595 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.47 s (2×CH_3), 1.87 t (*J*=7 Hz, $-CH_2-$), 2.72 t (*J*=7 Hz, $-CH_2-$), 7.90 m (2×Ar-H), 8.26 m (2×Ar-H); MS: *m/z* 242 $[M^+]$ ($C_{15}H_{14}O_3$). It converted to β-lapachone on treatment with conc. H_2SO_4.

Stigmasterol: Colourless needles, yield 1.0 g, m.p. 166-67°C (MeOH), R_f 0.34; IR (KBr): 3450 cm^{-1}; acetate, (Ac_2O / pyridine) m.p. 144-45°C; MS: *m/z* 412 $[M^+]$ ($C_{29}H_{48}O$).

β-Sitosterol: Colourless flakes, yield 1.5 g, m.p. 135-36°C (MeOH), R_f 0.33; IR (KBr): 3400 cm^{-1}; acetate, (Ac_2O / pyridine) m.p. 124-25°C; MS: *m/z* 414 $[M^+]$ ($C_{29}H_{50}O$).

β-Lapachone, 8: Red needles, yield 35 mg, m.p. 154-55°C, (*lit.*[23] 155-56°C), R_f 0.30; UV-Vis (EtOH): 256, 282, 330, 431 nm; IR (KBr): 1690, 1640, 1632, 1598 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.51 s (2×CH_3), 1.87 t (*J*=7 Hz, $-CH_2-$), 2.63 t (*J*=7 Hz, $-CH_2-$), 7.77 m (3×Ar-H), 8.22 m (1×Ar-H); MS: *m/z* 242 $[M^+]$ ($C_{15}H_{14}O_3$). It converted to α-lapachone on treatment with conc. HCl.

Computational Methodology: All *ab initio* MO calculations were performed using Gaussian 03M software package[20] running on a PowerMac G5 computer. Generation of the initial geometry was carried out using Chem3D molecular modeling software. Full geometry optimizations were done at the B3LYP/6-31G(d,p) level. The magnetic shielding were calculated with the GIAO method[15] at the MPW1PW91/6-31G(d,p) level[17]. The ^{13}C chemical shifts (δ ppm) were expressed for the difference from the isotropic shielding value of TMS (196.3280).

Acknowledgements

Authors thank the Council of Scientific and Industrial Research, New Delhi for awarding Emeritus Scientistship to one of us (P. Singh).

References

1 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian Medicinal Plants*, (CSIR, New Delhi), **1956**, 240.

2 Pandey V B & Gupta B D, *J Indian Chem Soc*, 48, **1971**, 937.

3 Seshadri T R, Gupta S R & Malik K K, *Indian J Chem*, 7, **1969,** 457.

4 Joshi K C, Prakash L & Singh P, *Phytochemistry*, 11, **1972,** 1498.

5 Joshi K C, Prakash L & Singh L B, *Phytochemistry*, 14, **1975**, 1441.

6 Joshi K C, Prakash L & Singh L B, *Phytochemistry*, 13, **1974,** 663.

7 Joshi K C, Singh P & Pardasani R T, *Planta Med*, 31, **1977,** 14.

8 Joshi K C, Sharma A K & Singh P, *Planta Med*, 1, **1986,** 71.

9 Joshi B S, Gawad D H, Pelletier S W, Kartha G & Bhandary K, *Tetrahedron Lett,* 25, **1984,** 5847.

10 Joshi B S, Jiang Q, Rho T & Pelletier S W, *J Org Chem*, 59, **1994,** 8220.

11 Simpson T J, *Natl Prod Rep*, 4, **1987**, 639.

12 Zassbi A R, Singh P, Jain S & Tahara S, *Helv Chim Acta,* 87,

2004, 820.

13 Barone G, Gomez-Paloma L, Duca D, Riccio R & Bifulco G, *Chemistry A European J*, 8, **2002,** 3233.

14 Scott K, Stonehouse J, Keeler J, Hwang T L & Shaka A J, *J Amer Chem Soc*, 117, **1995,** 4199.

15 Wolinski K, Hinton J F & Pulay P, *J Amer Chem Soc*, 112, **1990,** 8251.

16 Barfield M & Fagerness P, *J Amer Chem Soc*, 119, **1997,** 8699.

17 Adamo C & Barone V, *J Chem Phy*s, 108, **1998,** 664.

18 Rychnovsk S D, *Organic Lett*, 8, **2006,** 2895.

19 Bagno A, Rastrelli F & Saiell G, *Chemistry A European J*, 12, **2006,** 5514.

20 Frisch M J, Trucks G W, Schlegel H B, Scuseria G E, Robb M A, Cheeseman J R, Montgomery Jr J A, Vreven T, Kudin K N, Burant J C, Millam J M, Iyengar S S, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson G A, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M Li X, Knox J E, Hratchian H P, Cross J B, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann R E, Yazyev O, Austin A J, Cammi R, Pomelli C, Ochterski J W, Ayala P Y, Morokuma K, Voth G A, Salvador P, Dannenberg J J, Zakrzewski V G, Dapprich S, Daniels A D, Strain M C, Farkas O, Malick D K, Rabuck A D, Raghavachari K, Foresman J B, Ortiz J V, Cui Q, Baboul A G, Clifford S, Cioslowski J, Stefanov B B, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin R L, Fox D J, Keith T, Al-Laham M A, Peng C Y, Nanayakkara A, Challacombe M, Gill P M W, Johnson B, Chen W, Wong M W, Gonzalez C & Pople J A, *Gaussian 03,Revision B 4*, Gaussian, Inc, Wallingford CT **2004**.

21 Khanna R N, Sharma P K & Thomson R H, *J Chem Soc*, *Perkin Trans I,* **1987**, 1821.

22 Inoue K, Chen C C, Inouye H & Kuriyama K, *J Chem Soc*, *Perkin Trans I,* **1981**, 2764.

23 Burnett A R & Thomson R H, *J Chem Soc*, *Perkin Trans I,* **1967,** 2100.

24 Govindachari T R, Parthasarathy P C, Desai H K & Mohamed P A, *Indian J Chem*, 9, **1971,** 1027.