Rev. Colomb. Quím., 2010, **39**(2):173-180

SYNTHESIS AND ANTIOXIDANT ACTIVITY OF TWO ISOESPINTANOL DERIVATIVES

SÍNTESIS Y ACTIVIDAD ANTIOXIDANTE DE DOS DERIVADOS DE ISOESPINTANOL

SÍNTESE E ATIVIDADE ANTIOXIDANTE DOS DERIVADOS DO ISOESPINTANOL

Paula L. Galeano[1], Maritza A. Gil[1], Jesús H. Gil[1], León F. Otálvaro[2], Jairo A. Saéz[1], Benjamín A. Rojano[1,3]

Recibido: 29/04/10 – Aceptado: 18/08/10

ABSTRACT

The antioxidant activity of isoespintanol (1) hemisynthetic analogues, 4-bromo-2-isopropyl-3,6-dimethoxy-5-methylphenol (2) and 3-isopropyl-6-methylbenzene-1,2,4-triol (3), was evaluated using ABTS, DPPH and FRAP assays. Partial rationalization of the results is provided in terms of quantum chemical calculations of bond dissociation enthalpy (BDE) and ionization potential (IP).

Key words: isoespintanol, antioxidant activity, hemisynthesis, *Oxandra cf. xylopioides, Annonaceae*.

RESUMEN

La actividad antioxidante de los análogos hemisintéticos del isoespintanol (1), 4-bromo-2-isopropil-3,6-dimetoxi-5-metilfenol (2) y 3-isopropil-6-metilbenceno-1,2,4-triol (3), se evaluó empleando los ensayos ABTS, DPPH y FRAP. La racionalización de los resultados es provista de forma parcial en términos de cálculos cuánticos de entalpía de disociación de enlace (BDE) y potencial de ionización (IP).

Palabras clave: isoespintanol, actividad antioxidante, DPPH, ABTS, FRAP, hemisíntesis, *Oxandra cf. xylopioides*.

RESUMO

A atividade antioxidante dos análogos hemi-sintéticos de isoespintanol (1), 4-bromo-2-isopropil-3,6-dimetoxi-5-metilfenol (2) e 3-isopropil-6-metilbenzeno-1,2,4-triol (3) foi avaliada através das técnicas de ABTS, DPPH e FRAP. A ra-

1 Laboratorio Ciencia de los Alimentos, Facultad de Ciencias, Universidad Nacional de Colombia, sede Medellín, Calle 59A No. 63-20, Medellín, Colombia.

2 Instituto de Química, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Calle 67 No. 53-108, Medellín, Colombia.

3 brojano@unal.edu.co

cionalização dos resultados está prevista, em parte, em termos de cálculos quânticos de entalpia de dissociação da ligação (BDE) e potencial de ionização (IP).

Palavras-chave: isoespintanol, atividade antioxidante, DPPH, ABTS, FRAP hemi-síntese, *Oxandra* cf. *xylopioides*.

INTRODUCTION

Isopropylphenols represent an important class of compounds to which human beings are constantly exposed. They are employed in different applications including flavoring additives, fragrances, antiseptics, fungicides and antioxidants. For example, *propofol* (2,6-diisopropylphenol) is an intravenous short-action anesthetic with minimum side effects, controllable anesthetic state and fast general anesthesia. It has been also reported as antioxidant, immunomodulator, carbonic anhydrase enzyme inhibitor and neuroprotective (1, 2). Thymol (5-methyl-2-isopropilphenol) and carvacrol (5-isopropyl-2-metilphenol), isolated from the essential oil of Lamiaceae plants, are reported as promising antioxidants capable of inhibiting the lipid peroxidation of phospholipidic liposomes in a concentration dependent way (3). Moreover, thymol and carvacrol have been reported as antiseptic and fungicide respectively (4).

Isoespintanol (2-isopropyl-3,6-dimethoxy-5-methylphenol) (1), is a crystalline monoterpene isolated from *Oxandra cf. xylopiodes* (Annonaceae) leaves in a 1.5% yield, with antioxidant activity (5, 6). DPPH and FRAP assays for this substance indicate that it is twice more active than typical antioxidants, such as thymol. This effect is attributed to the molecule substituents and the intra and intermolecular hydrogen interactions (7). Isoespintanol (1), being a pentasubstituted benzene, can be anticipated as a recalcitrant substrate for electrophylic aromatic substitution as any reaction of this type will end up with a fully substituted benzene ring. Our initial attempts to introduce a versatile nitro group were frustrated by the harsh conditions needed in order to obtain an almost negligible yield. Therefore, the introduction of others substituents in the aromatic ring or deprotection of methoxyl groups in isoespintanol seems to be a promising strategy for the enhancement of the antioxidant activity of the molecule.

Here we report on the hemisynthesis and subsequent evaluation of the antioxidant activity of two analogues of isoespintanol, namely, 4-bromo-2-isopropyl-3, 6-dimethoxy-5-methylphenol (2) and 3-isopropyl-6-methylbenzene-1,2,4-triol (3). Quantum chemical calculations of bond dissociation energy (BDE) and ionization potential (IP) are offered as a means of rationalization.

MATERIALS AND METHODS

General

NMR analysis (1D and 2D) were conducted on a Bruker AMX 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) spectrometer with a direct inversion prove. TMS was used as internal standard. EI-Mass analysis were conducted on an Agilent 6890N GC coupled to an Agilent 5973N Mass Selective Detector operating at 70 eV. Helium was used as carrier gas. IR and UV Spectra were measured on a Perkin-Elmer RX I FT-IR system (KBr disk) and Jenaway

6405 spectrophotometer respectively. Melting points were measured on a Büchi apparatus (serial: 510218) are uncorrected.

Milli –Q– water (Millipore, Bedford, MA) was used in all work, ABTS (2,2 0-azinobis-(3-ethylbenzothiaoline-6-sulphonic-acid), Trolox-(6-hydroxy-2, 5, 7,8 tetra-methyl-chroman-2-carboxylic-acid), 2,2 diphenyl-1-picryl hydrazyl (DPPH$^{\bullet}$) and TPTZ (2,4,6-tripyr-idyl-S-triazine) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate, potassium persulphate, L -ascorbic acid, $FeCl_3.6H_2O$ and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany), methanol and other solvents were purchased from Fisher Scienti?c Co. (Fair Lawn, NJ, USA).

Bromination and demethylation of isoespintanol

The method of Majetich et al. was adapted in this case (8). 500 mg (2,38 mmol) of isoespintanol (1) were dissolved in DMSO (15 mL) in a 50 mL round bottomed flask equipped with a magnetic stirrer. Careful dropwise addition of HBr (48%, 5 mL) followed during a period of 10 minutes. The mixture was allowed to stir for 48h at 25 °C before partition with CH_2Cl_2-H_2O (30 mL each) The organic phase was distilled at reduced pressure and submitted to silica gel column chromatography (Kisielgel 60, Merck 0.063-0.200 mesh) using *n*-hexane: ethyl acetate (95:5) as mobile phase to afford 4-bromo-2-isopropyl-3,6-dimethoxy-5-methylphenol (2) and 3-isopropyl-6- methylbenzene-1,2,4-triol (3) in 30% and 65% isolated yield respectively.

4-bromo-2-isopropyl-3,6-dimethoxy-5 -methylphenol (2)

Red solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 1.34 (d, J= 7.2 Hz, 3H, H-8), 1.37 (d, J= 7.2 Hz, 3H, H-9), 2.33 (s, 3H, H-10), 3.44 (m, 1H, H-7), 3.75 (s, 3H, H-11), 3.78 (s, 3H, H-12), 5.80 (s, 1H, OH). **^{13}C NMR** (100 MHz, $CDCl_3$,) δ 16.4 (C-10), 20.8 (C-8 y C-9), 26.6 (C-7), 61.2 (C-11), 61.4 (C-12), 110.4 (C-4), 126.6 (C-2), 128.5 (C-5), 142.6 (C-3), 147.5 (C-5). **EI-MS** m/z 288 (97), 290 (94), 273 (20), 275 (19). **IR** (KBr, cm^{-1}) 3412, 1638, 1125, 1197 C-Br ext, cm^{-1}. **UV** (MeOH) λ_{max} nm (log ε): 205 (3.82), 250 (3.28), 270 (3.42), 335 (3.36). mp: 47-48 °C.

3-isopropyl-6-methylbenzene-1, 2,4-triol (3)

Orange solid. **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 1.13 (d, J= 7.2 Hz, 3H, H-8), 1.14 (d, J= 7.2 Hz, 3H, H-9), 2.08 (s, 3H, H-10), 3.20 (m, 1H, H-7), 6.48 (s, 1H, H-5), 6.96 (s, 2H, OH, C-2 y C-4), 7.28 (s, 1H, C-1). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 15.0 (X−10), 20.2 (X−8 ψ X−9), 24.5 (X−7), 125.9 (X−5), 136.2 (X−3), 136.4 (X−6), 140.9 (X−2), 51.2 (X−4), 187.7 (X−1). **EI-MS** m/z 182 $[M]^+$ (5), 180 (100), 165 (25), 137 (47). **IR** (KBr, cm^{-1}) 3238, 1615, 1282, 1197 cm^{-1}. **UV** (MeOH) λ_{max} nm (log ε): 210 (3.84), 230 (3.60), 265 (4.30), 405 (3.33). mp: 175-176 °C.

Antioxidant activity

TEAC DPPH assay

Radical scavenging activity against the stable radical DPPH was measured using the methods of Brand-Williams et al. with

some modifications as described below (7). Compounds were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. A volume of 990 μl from a 10 μM DPPH methanol solution was added to 10 μl of solution and allowed to react at room temperature. After 30 min the absorbance (time to reach the stationary phase) values were measured at 517 nm. The results were expressed as μmol Trolox equivalent per 100 g of compound using a Trolox standard curve (50-100 μM).

FRAP assay

The procedure described by Benzie and Strain was followed with some modifications as described below (7). The principle of this method is based on the increasing in absorbance due to the formation of the complex 2,4,6-tripyridil-*s*-triazine (TPTZ)-Fe (II) in the presence of reducing agents. The FRAP reagent contained 2,5 mL of a 10 μM TPTZ in 40 mM HCl plus 2,5 mL of 20 μM $FeCl_3$ and 25 mL of 0,3 μM acetate buffer, pH 3.6 and was prepared freshly and warmed at 37 C. A volume of 50 μl solution were mixed with 50 μl acetate buffer, pH 3,6 and 900 μL FRAP reagent. The absorbance increase was measured at 593 nm. The FRAP value were expressed as AEAC (Ascorbic Acid Equivalent Antioxidant Capacity: mg ascorbic acid per 100 g of compound) using an ascorbic acid standard curve (50-100 μM).

TEAC ABTS assay

The radical was prepared for the oxidation reaction of 3,5 mM ABTS solution with 125 mM potassium persulfate solution. The $ABTS^{\bullet+}$ radical solution was diluted with phosphate buffer 10 mM to obtain an absorbance of $0,7 \pm 0,005$ units at 732 nm using the spectrophotometer. A volume of 990 μl from $ABTS^{\bullet+}$ solution was added to 10 μl of solution and allowed to react at room temperature, after 30 min (time to reach the stationary phase) the absorbance values were measured at 732 nm (9). The results were expressed as μmol Trolox equivalent per 100 g of compound using a Trolox standard curve (50-100 μM).

All analyses were done in quadruplicate, and all compounds tested were dissolved in methanol. Regressions were calculated with a significance level of 95% ($p < 0.05$) using Statgraphics Plus 5.0 (Statistical Graphics Corp., Rockville, MD).

Computational methods

Gas phase bond dissociation enthalpies (BDE) and ionization potentials (IP) were obtained using the full basis calculation method reported by Wright et al. (10). All calculations were performed within *Spartan´08* (*Spartan´08* Wave function, Inc. Irvine, CA). BDE and IP values were compared with the values for phenol and are reported as ΔBDE and ΔIP defined as $\Delta BDE = BDE\ (O\text{-}H)_{(x)} - BDE\ (O\text{-}H)_{(phenol)}$; $\Delta IP = IP_{(x)} - IP_{(phenol)}$; Gas phase BDE and IP computational values for phenol were 87.10 Kcal/mol and 188.78 Kcal/mol respectively.

RESULTS AND DISCUSSION

In situ generation of bromodimethylsulfonium bromide (HBr/DMSO) is considered a mild method for the electrophilic

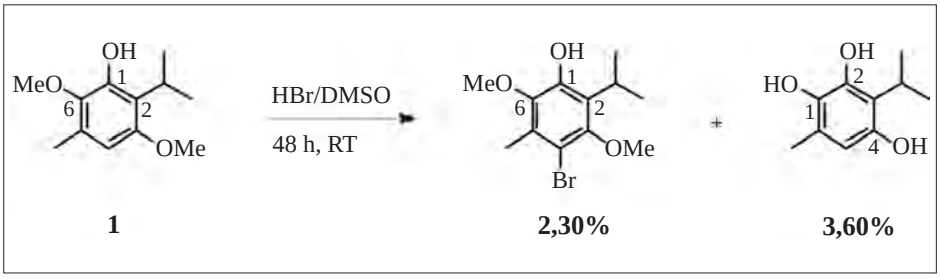

Figure 1. Bromination and demethylation of isoespintanol with HBr/DMSO.

bromination of arenes (8). In our case, this allowed the introduction of the bromine atom into isoespintanol (1) only as a side reaction, demethylation been the major transformation under these conditions (Figure 1). Products 2 and 3 (Figure 1) were easy to separate making this reaction a useful process in terms of rapid structural diversification. The lowest yield of brominated compounds (30%) may be due to the size of bromine, which is sterically hindered, demethylation reactions to be favored (60%) by acidic conditions of the environment.

Previous work with isoespintanol (1) (5, 7), suggested that the antioxidant activity of 1 was highly influenced by the substitution pattern of the phenyl ring and the hydrogen bonds present in such molecule; therefore, the structural characteristics of compounds 2 and 3 raised questions about how the newly introduced groups will affect the antioxidant activity relative to isoespintanol (1).

Table 1 display the results of ABTS, DPPH and FRAP analyses for compounds 2 and 3. Results for isoespintanol (1), thymol and butylated hydroxyltoluene (BHT) are also presented for reference purposes. Several tendencies were observed, for example, the ABTS assay afforded activities for the tested compounds in the following order 1 > 2 > 3, which contrast with the activities observed in the DPPH (1 > 3 > 2) and FRAP assays (3 > 1 > 2). Interestingly, the FRAP assay afforded compound 3 as the most active compound with an activity higher than

Table 1. ABTS, DPPH and FRAP assays for isoespintanol and nalogues.

Compound	ABTS TEAC (μM Trolox/mmol	DPPH TEAC (μM Trolox/mmol	FRAP AEAC (mg AA/100g
1	1098 ± 50	23 ± 1	66 ± 4
2	564 ± 25	6.4 ± 0.5	73 ± 3
3	121 ± 6	15 ± 4.0	15 ± 1
Thymol	1343 ± 55	10.5 ± 1.0	27 ± 1
BHT	1604 ± 62	175.1 ± 5.4	30 ± 2

Table 2. (RO)B3LYP/6-311+G(2d,2p)//AM1/AM1 and (U)B3LYP/6311G(d)//AM1/AM1calculated ΔBDE and ΔIP values in the gas phase for compounds 1-3 (All values in Kcal/mol).

Compound	ΔBDE		ΔIP
1	-3.9		-32.0
2	-4.0		-19.1
3	-12.0 (C2-O-H bond)	-4.4 (C4-O-H bond)	-28.0

BHT or thymol, however, this phenomenon was not observed in other assays. It is well accepted that bond dissociation enthalpies (BDE´s) and ionization potentials (IP´s) are important factors in determining the efficacy of an antioxidant (10); therefore, theoretical calculations were performed in an attempt to correlate the order of activities with these two parameters. Table 2 presents these results.

BDE calculations for compound 3 deserves special mention for in this case it is easy to assume that the two catechol type hydroxyls attached at C1 and C2 can generate a single hydrogen bonded stabilized radical (Figure 2) and thus having the lowest BDE value compared to the hydroxyl attached at C4 for the same compound (Figure 2). Calculations showed this to be the case, however, C4-O-H bond in compound 3 can be more accessible for kinetic reasons and therefore we decided to keep also this value into consideration (Table 2). DPPH and FRAP assays are generally classified as single electron transfer (SET) reactions (11) and

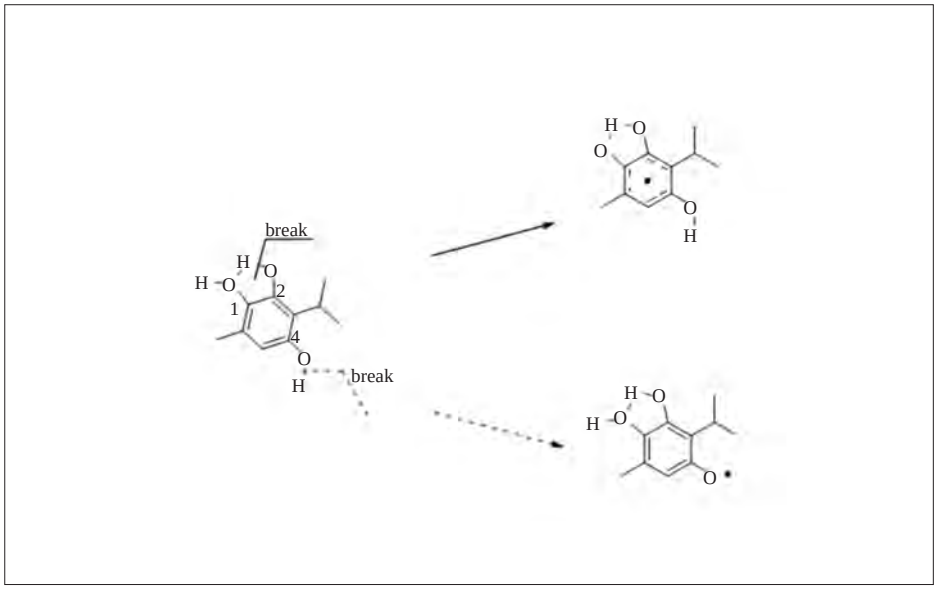

Figure 2. Parent compound 3 and radicals formed by H-atom transfer (HAT) at two distinct sites.

therefore, a correlation with ΔIP can be expected for this assays. Interestingly, the predicted order of activity according to ΔIP (Table 2) would be 1 > 3 > 2 which parallels the DPPH assay but not completely the FRAP assay. However, it seems clear that the bromine substituent lowers the energy of the highest occupied molecular orbital (HOMO) disfavoring a SET mechanism. Interestingly, according to BDE calculations (Table 2), the order of activity would be 3 > 2 ~ 1 if an H-atom transfer (HAT) mechanism is assumed, however, this tendency was not observed in the ABTS assay. Therefore, it is likely that a HAT mechanism is not the predominant one for compounds 1-3 under the reaction conditions explored. It is worth to notice that the ABTS assay cannot be classified as a predominantly HAT reaction (11) and therefore, a more specific HAT assay like the oxygen radical absorbance capacity (ORAC) is desirable in this case (12). BDE values for compounds 1 and 2 are similar; therefore, similar reactivity is expected. However, reducing capacity for compound 1 is lower in DPPH and ABTS assays, which may be due to steric hindrance caused by bulky bromine atom does not lead to the active sites of the reagents DPPH• and ABTS• respectively. Compound 3 possesses a high solubility in water; therefore it is dispersed throughout the aqueous medium favoring the formation of intra and intermolecular hydrogen bonds, decreasing the reducing activity of compound 3 in the ABTS and FRAPS assays.

In general, these results indicate that the introduction of a cathecol moiety could favor a HAT mechanism in isoespintanol like compounds. This in conjunction with the introduction of a bromine atom (which seems to disfavor a SET mechanism) offers the possibility of designing molecules that predominantly work by HAT. We postulate that a molecule like 4-bromo-6-isopropyl-5-methoxy-3-methylbenzene-1,2-diol could be one such candidate and this work is currently under way.

ACKNOWLEDGEMENT

This work was financially supported by Dirección de Investigaciones Universidad Nacional de Colombia, sede Medellín (DIME) – Jóvenes investigadores e innovadores de Colciencias – Francisco José de Caldas, Grant number 20101007684 and Universidad de Antioquia (programa de sostenibilidad).

REFERENCES

1. Gelb, A. W.; Bayona, N. A.; Wilson, J. X.; Cechetto, D. F. Propofol anaesthesia compared to awake reduces infarct size in rats. *Anesthesiology*. 2001. **96**: 1183–1190.

2. Engelhard, K.; Werner, C.; Eberspacher, E.; Pape, M.; Stegemann, U.; Kellermann, K.; Hollweck, R.; Hutzler, P.; Kochs, E. Influence of propofol on neuronal damage and apoptotic factors after incomplete cerebral ischemia and reperfusion in rats: a long-term observation. *Anesthesiology*. 2004. **101**: 912–917.

3. Aeschbach, R.; Loé Liger, J.; Scott, B. C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O. T. Antioxidant action of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol.

Food Chem. Toxicol. 1994. **32**: 31-36.

4. Menphini, A.; Pagiotti, R.; Capuccella, M. Antifugal activity of carvacrol chemotypes of winter savory harvested in Italy. *Riv. Ital. EPPOS*. 1993. **4**: 566- 571.

5. Rojano, B.; Pérez, E.; Figadére, B.; Martin, M. T.; Recio, M. C.; Giner, R.; Ríos, J. L.; Schinella, G.; Sáez. J. Constituents of Oxandra cf. xylopioides with Anti-inflammatory Activity. *J. Nat. Prod*. 2007. **70**: 835-838.

6. Rojano, B.; Gaviria, C.; Gil, A.; Sáez, J.; Schinella, G.; Tournier. H. Actividad antioxidante del isoespintanol en diferentes medios. *Vitae*. 2008. **15**(1): 173-181.

7. Rojano, B.; Otiz, E.; Gil, M.A.; Notario, R.; Schinella, G.; Saéz, J. And Quijano, J. Experimental and Theoretical Determination of the Antioxidant Properties of Isoespintanol (2-Isopropyl-3,6-dimethoxy-5-methylphenol). *J. Mol. Struct*. 2008. **877**: 1-6.

8. Majetich, G.; Hicks, R.; Reister, S. Electrophilic aromatic bromination using bromodimethylsulfonium bromide generated in situ. *J. Org. Chem.* 1997. **62**: 4321-4326.

9. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio. Med.* 1999. **26**: 1231-1237.

10. Wright, J.; Jhonson, E.; Dilabio, G. Predicting the Activity of Phenolic Antioxidants: Theoretical Method, Analysis of Substituent Effects, and Application to Major Families of Antioxidants. *J. Am. Chem. Soc.* 2001. **123**: 1173-1183.

11. Prior, R.; Wu, X.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food. Chem.* 2005. **53**: 4290-4302.

12. Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food. Chem.* 2005, **53**: 1841-1856.