Nitrosation Kinetics of Phenolic Components of Foods and Beverages

M. PAZ FERNÁNDEZ-LIENCRES,* EMILIO CALLE, SAMUEL GONZÁLEZ-MANCEBO, and JULIO CASADO†

Departamento de Química física, Facultad de Química, Universidad de Salamanca, E-37008, Salamanca, España

BARTOLOMÉ QUINTERO

Departamento de Química física, Facultad de Farmacia, Universidad de Granada, E-18071, Granada, España

ABSTRACT

The kinetics of the reactions between sodium nitrite and phenol or *m*-, *o*-, or *p*-cresol in potassium hydrogen phthalate buffers of pH 2.5–5.7 were determined by integration of the monitored absorbance of the C-nitroso reaction products. At pH > 3, the dominant reaction was C-nitrosation through a mechanism that appears to consist of a diffusion-controlled attack on the nitrosatable substrate by $NO^+/NO_2H_2^+$ ions followed by a slow proton transfer step; the latter step is supported by the observation of basic catalysis by the buffer which does not form alternative nitrosating agents as nitrosyl compounds. The catalytic coefficients of both anionic forms of the buffer have been determined. The observed order of substrate reactivities (*o*-cresol $\approx$ *m*-cresol $>$ phenol $\gg$ *p*-cresol) is explained by the hyperconjugative effect of the methyl group in *o*- and *m*-cresol, and by its blocking the *para* position in *p*-cresol. Analysis of a plot of $\Delta H^{\#}$ against $\Delta S^{\#}$ shows that the reaction with *p*-cresol differs from those with *o*- and *m*-cresol as regards the formation and decomposition of the transition state. The genotoxicity of nitrosatable phenols is compared with their reactivity with $NO^+/NO_2H_2^+$.

INTRODUCTION

The nitrosonium ion NO^+ being some 10^{14} times less reactive than the nitronium ion NO_2^+ [1,2] much less attention has been paid to the C-nitrosation of aromatics than to their nitration, which is now well understood [3]. The nitrosation of certain aromatics is nevertheless of considerable biomedical interest in view of the proven carcinogenic or mutagenic properties of the resulting products. This is the case of phenol and its derivatives [4–7]: the phenol derivative tyramine, which occurs in cheese, meat extract, beer, and soybean products [8–10] has been identified as one of the precursors largely responsible for the mutagenic activity of certain Japanese soy sauces treated with nitrite [11,12]; and bamethan [1-(4-hydroxyphenyl)-2-butylaminoethan-ol], a phenolic drug used for long-term oral treatment of cardiovascular disease, is both nitrosatable and a directly acting mutagen [13]. In view of their status as nitrosatable pre-

Received May 8, 1995; accepted March 5, 1996

* On leave from Departamento de Química física y Analítica, Facultad de Ciencias Experimentales, Universidad, E-23071, Jaén, Spain.

† Author to whom correspondence should be addressed.

International Journal of Chemical Kinetics, Vol. 29, 119–125 (1997)
 CCC 0538-8066/97/020119-07

cursors of genotoxic substances, it is of some concern that phenol, catechol, vanillin, and other phenolics have been detected in smoked fish and meats [14]. It has also been reported that the presence of phenolic compounds can block the *N*-nitrosation reactions of other substrates [15,16], and although this has been questioned on the grounds that *N*-nitrosation is catalyzed by monohydroxy phenols [17] (but not by all polyhydroxyphenols [15]), it should be borne in mind that catalytic activity is only observed when the concentration of nitrosating agent significantly exceeds the concentration of phenol, which rarely occurs in vivo or in environmentally significant situations.

In spite of the biomedical significance of the nitrosation of phenolics, little research has been done on the mechanisms of these reactions [18]. However, according to Challis and co-workers [1,19–21] the C-nitrosation of simple phenols in perchloric media generally involves the formation of a dienone intermediate that loses a proton from its nitrosated carbon to yield the nitrosophenol in equilibrium with its oxime form. *p*-Alkylphenols yield the corresponding 2-nitrosocompounds, whereas *p*-bromophenol is transformed by a fast substitution reaction into *p*-nitrosophenol, although in both cases the nitrosocompound is rapidly oxidized to the corresponding nitrocompound.

In the case of the nitrosation in perchloric media the 2-nitrosocompounds are considered to be rapidly oxidized to the corresponding nitrocompound. However, the course of the reaction between nitrite and *p*-methoxyphenol appears to depend on the concentration of perchloric acid in the medium: 4-methoxy-2-nitrophenol is only formed if the perchloric acid concentration is low (pH 2–3), and even then it is not the major product; if perchloric acid concentration is high, benzoquinone is formed in quantitative yield [20]. Similar results for phenol and 4-phenoxyphenol as well as 4-methoxyphenol were obtained by Moodie and co-workers [22–24] in studies that, like others on the nitrosation of certain phenolic drugs [13,25–28], also showed that the course of these reactions can depend on the concentration of dissolved oxygen in the medium and on whether the concentration of nitrite is greater or less than that of the substrate.

While different studies have been carried out in buffered media by buffers such as acetic-acetate or monochloroacetic-monochloroacetate ([29]; Challis et al., loc. cit.), practically no work has been done with buffers which do not form alternative nitrosating agents in the form of nitrosyl compounds.

In this work we investigated the nitrosation of phenol and of *m*-, *o*-, and *p*-cresol by sodium nitrite in potassium hydrogen phthalate buffers that did not form any potential nitrosating agent.

EXPERIMENTAL

Phenol and *m*-, *o*-, and *p*-cresol were Aldrich p.a. products (Steinheim, Germany). Solutions of $NaNO_2$, $HClO_4$, and NaOH (all Merck p.a. products, Darmstadt, Germany) and of potassium hydrogen phthalate (Panreac p.a., Barcelona, Spain) were made up by weight ($NaNO_2$ solutions after desiccation for 2 h at 110°C). Reaction mixtures were made up in potassium hydrogen phthalate buffers of pH 2.5–5.7. pH was measured with a Radiometer M64 pH-meter equipped with a GK2401B combined electrode.

Reaction kinetics were followed in a Shimadzu 2101PC Vis-UV spectrophotometer by monitoring absorbance by the nitrosated product at 345 nm, an isosbestic point at which there is no interference by the reagents (Fig. 1). To 3 cm^3 of substrate solution in a cell thermostatted at 25 ± 0.1°C was added 0.1 cm^3 of sodium nitrite solution to a concentration of 1 10^{-4} M (very much less than the initial substrate concentrations: 0.030–0.200 M for phenol, between 5 10^{-3} and 6 10^{-2} M for *o*- and *m*-cresol, and 0.040–0.125 M for *p*-cresol). The absorbance-time data were processed by the integration method; all reactions were followed to at least 70% completion.

RESULTS AND DISCUSSION

Spectrum of *p*-nitrosophenol at various pH values is shown in Figure 1. It can be observed the appearance

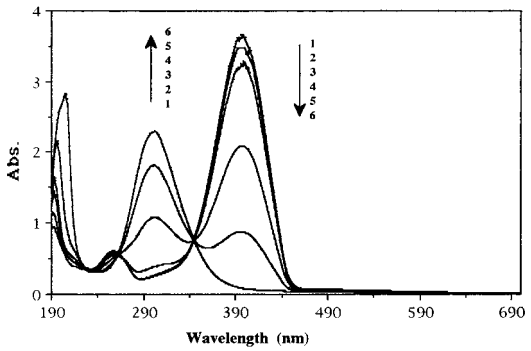


Figure 1 UV Spectra of *p*-nitrosophenol (1.38×10^{-4} M, T = 298 K, and I = 0.20 M) under various pH conditions: (1) pH = 12.16; (2) pH = 10.77; (3) pH = 7.49; (4) pH = 6.49; (5) pH = 5.78; and (6) pH = 2.53.

of an isosbestic point at 345 nm attributed to an equilibrium between the tautomeric forms (benzoquinone mono-oxime and *p*-nitrosophenol) and the *p*-nitrosophenolate anion, pK_a = 6.5 [30].

Kinetic experiments were carried out in potassium hydrogen phthalate buffers, which were found not to react with the nitrosating agent. Plots of $\ln(A_\infty - A_0)/(A_\infty - A_t)$ against time were linear, showing the reactions to be of first-order with respect to nitrite:

$$v = k_{1\text{exp}}\,[\text{NaNO}_2] \qquad (1)$$

It must be pointed out that the first-order respect to nitrite indicates that the nitrosation by N_2O_3 is not involved in this mechanism. Reaction is believed to occur by the attack of nitrosatable substrate by $NO_2H_2^+/NO^+$ ions kinetically indistinguishable.

The corresponding first-order pseudoconstants were calculated from the slopes of these plots. Their logarithms depended linearly on the logarithm of substrate concentration (Fig. 2), allowing the second-order coefficients $k_{2\text{exp}}$ to be calculated:

$$\text{rate} = k_{2\text{exp}}\,[\text{nitrite}]\,[\text{substrate}] \qquad (2)$$

These $k_{2\text{exp}}$ were then transformed into corrected values $k_{2\text{corr}}$ that took into account the dissociation of nitrous acid (pK 3.15 [31]):

$$k_{2\text{corr}} = k_{2\text{exp}}\,(1 + K_{\text{HNO}_2}/[\text{H}^+]) \qquad (3)$$

There was no need to consider dissociation of the substrates, all of which have pK values very much higher than the pH range used in this work: pK (phenol) = 10.00, pK(*o*-cresol) = 10.29, pK(*m*-cresol) = 10.09, and pK(*p*-cresol) = 10.26 [32].

The fact that for all the substrates $k_{2\text{corr}}$ peaked at an acidity of about pH 3.5 (Table I) suggests that the reaction mechanism was the same in all cases. In fact,

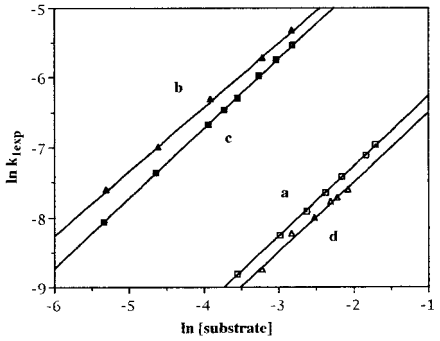


Figure 2 Influence of the concentration of substrate upon the pseudo-first-order rate constant of its nitrosation. pH = 3.5; [nitrite] = 10^{-4} M; T = 310 K (T = 298 K for phenol); I = 0.10 M; (a) phenol; (b) *o*-cresol; (c) *m*-cresol; and (d) *p*-cresol.

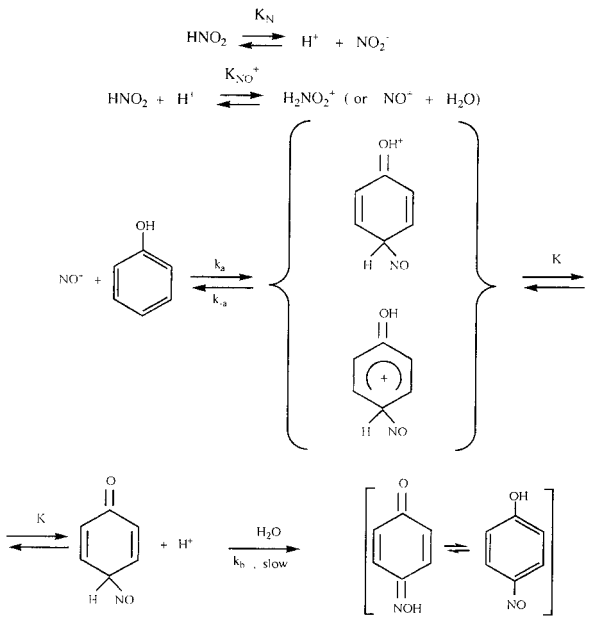
the observed data are explicable in terms of aromatic electrophilic substitution by nitrosonium or nitrous acidium ions (Scheme I), a mechanism previously suggested by other studies of the nitrosation of phenols by nitrous acid [19,33], and which parallels the mechanism proposed for the nitrosation of phenols [34] and species such as sulphamic acid and cysteine [35] by alkyl nitrites in aqueous acid solution. This mechanism implies the rate equation

$$\text{v} = \frac{k_\text{a}\,K_{\text{NO}^+}[\text{nitrite}][\text{phenol}][\text{H}^+]^2}{(K_\text{N} + [\text{H}^+])\left(1 + \dfrac{k_{-\text{a}}}{Kk_\text{b}}[\text{H}^+]\right)} \qquad (4)$$

The slow step in Scheme I is hypothesized, following Challis [1], to involve protonation of the nitroso oxygen in the transition state (Scheme II). Since this implies susceptibility to basic catalysis by organic bases, Schemes I and II are supported by the finding that, for phenol, $k_{2\text{corr}}$ depends linearly on buffer concentration, $[\text{B}]_T$:

Table I Values of $k_{2\text{corr}} \times 10^2$, $\text{M}^{-1}\,\text{s}^{-1}$ in the Nitrosation of Phenol and Related Compounds in Solutions of Hydrogen Phthalate Buffers: [Nitrite] = 10^{-4} M; [Phenol] = 0.20 M; [*o*- and *m*-Cresol] = 0.06 M; [*p*-Cresol] = 0.125 M; and T = 310 K

pH	Phenol	pH	*o*-Cresol	pH	*m*-Cresol	pH	*p*-Cresol
2.59	3.44	2.58	20.2	2.59	16.6	2.56	0.700
2.99	3.99	2.99	23.9	3.00	18.0	2.99	1.03
3.75	5.43	3.51	26.7	3.51	22.1	3.51	1.66
4.01	5.39	3.71	24.7	3.72	20.9	3.74	2.03
4.55	3.22	4.00	22.8	3.97	20.0	3.97	1.80
5.10	2.29	4.48	21.1	4.49	17.1	4.45	1.27
5.53	1.09	5.07	20.2	5.07	9.10	5.04	1.09

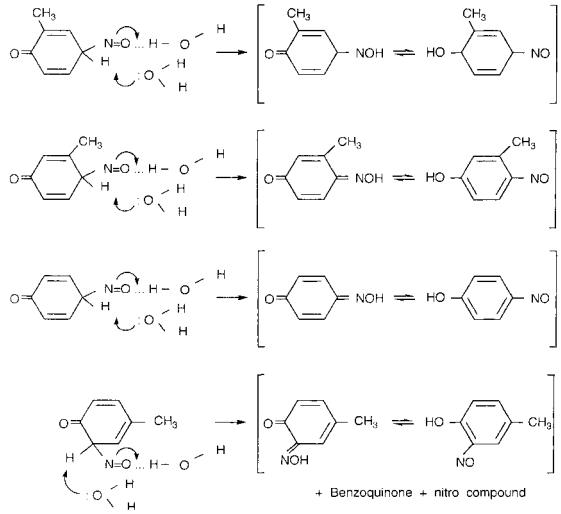


Scheme I

$$k_{2\text{corr}} = k_{2H_2O} + k_B[B]_T \quad (5)$$

where k_{2H_2O} is the rate constant for the uncatalyzed reaction, k_B the buffer catalytic constant, and $[B]_T = [BH_2] + [BH^-] + [B^=]$.

The increase in reaction rate with buffer concentration was indeed due to general basic catalysis rather than to the formation of more effective nitrosating agents in the form of nitrosyl compounds, because no nitrosyl compounds were detected (whereas in other media they have been detected and the equilibrium constants of their formation and hydrolysis have been measured [36,37]). Our findings



Scheme II

Table II Catalysis by Hydrogen Phthalate Buffers and Rate Constants of the Nitrosation of Phenol[a]

pH	k_B, $M^{-2}\,s^{-1}$	$k_{2H_2O}\,10^3$, $M^{-1}\,s^{-1}$
2.46	0.109	10.7
2.88	0.133	10.8
3.29	0.151	10.1
3.52	0.181	9.99
3.76	0.162	9.37
4.22	0.179	7.76
4.55	0.171	6.66
4.81	0.179	5.36
5.24	0.159	2.78
5.64	0.156	1.73

[a] [Phenol] = 0.20 M; [Nitrite] = 10^{-4} M; I = 0.20 M ($NaClO_4$); and T = 298 K.

therefore confirm the suggestion of Castro et al. [29] on the basis of results obtained in acetic acid/acetate buffers. The values of k_{2H_2O} and k_B determined at various pH are listed in Table II.

Identification of eqs. (2) and (4) implies

$$k_{2H_2O} = \frac{k_a K_{NO^+}[H^+]}{\left(1 + \dfrac{k_{-a}}{Kk_b}[H^+]\right)} \quad (6)$$

Fitting eq. (6) to the experimental data by means of an optimization algorithm [38,39] (see Fig. 3) yields the value $k_aK_{NO^+} = 661 \pm 48\ M^{-2}s^{-1}$ and $k_{-a}/Kk_b = 63.183 \pm 5.000\ M^{-1}$.

Similar pH dependence has been reported for the nitrosation of 2-naphtol [21] (those experiments were carried out in dilute $HClO_4$, but since ClO_4^- does not catalyze the reaction, the observed pH dependence is comparable with the pH dependence of k_{2H_2O} in our work).

Since $K_{NO^+} = 3 \times 10^{-7}\ M^{-1}$ [40], k_a value has been calculated to be $k_a = (2.2 \pm 0.2) \times 10^9\ M^{-1}s^{-1}$. That means the aromatic electrophilic substitution shown in Scheme I is diffusion-controlled like for other nitrosation reactions by $NO_2H_2^+/NO^+$ [41–44].

Having taken into account that, in the presence of the phthalate buffer

$$k_{2\text{corr}} = k_{2H_2O}(1 + k_{BH^-}[BH^-] + k_{B^=}[B^=]) \quad (7)$$

it is easy to demonstrate that

$$k_{2\text{corr}} = k_{2H_2O}\left\{1 + \left(\frac{k_{BH^-}K_1[H^+] + k_{B^=}K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2}\right)[B]_T\right\} \quad (8)$$

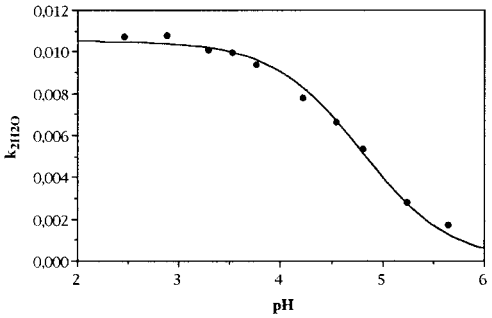


Figure 3 Variation of the second-order rate constant with pH for the nitrosation of phenol. The curve represents the dependence on pH calculated from eq. (6) and the points represent the observed rate constants (extrapolated to zero phthalate buffer concentration at pH = 2.5–5.7). Experimental conditions in Table II.

where

$$K_1 = [BH^-][H^+]/[BH_2]$$
$$K_2 = [B^=][H^+]/[BH^-] \quad (9)$$

Comparing eqs. (5) and (8) it results

$$\frac{k_{2H_2O}}{k_B[H^+]} = \frac{[H^+]/K_1 + 1 + K_2/[H^+]}{k_{BH^-}[H^+] + k_{B^=}K_2} \quad (10)$$

Figure 4 shows the experimental data fit well to eq. (8). Given that $K_1 = 1.3 \times 10^{-3}$ and $K_2 = 3.9 \times 10^{-6}$ [45] catalytic coefficients can be calculated, $k_{BH^-} = 13 \pm 6\ M^{-1}$ and $k_{B^=} = 132 \pm 13\ M^{-1}$.

Almost throughout the pH range used in this work, the substrate reactivities indicated by the observed values of k_{2corr} exhibited the order *o*-cresol ≈ *m*-cresol > phenol ≫ *p*-cresol. That *o*- and *m*-cresol are more reactive than phenol is attributable to the hyperconjugative effect of ortho and meta methyl groups [46], which may be expected to facilitate the transfer of a proton from water to the —NO group of the dienone intermediate (Scheme II). The poor reactivity of *p*-cresol may be attributed fundamentally to the occupation of the preferred para position by the methyl group, which forces nitrosation to occur at the less reactive ortho position.

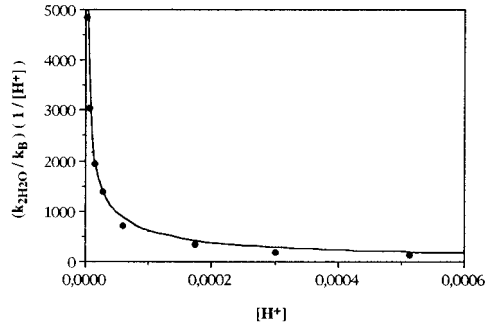


Figure 4 Dependence of $k_{2H_2O}/k_B[H^+]$ on $[H^+]$ in the nitrosation of phenol at 298 K and I = 0.20 M in the presence of potassium hydrogen phthalate buffer.

At first sight, the above order of reactivities seems to contradict the findings of Rosenkranz et al. [47], who in their studies of the genotoxicity of nitrosatable phenols and their derivatives found confirmation of the order *p*-cresol > phenol > *o*-cresol ≈ *m*-cresol predicted by an artificial intelligence system for the investigation of structure-activity relationships. It should be borne in mind, however, that in the conditions considered by Rosenkranz et al. (presence of excess nitrite, which as mentioned in the Introduction is in any case an unlikely natural situation) the intermediate reaction products are phenyldiazoniums rather than, as in our work, nitrosocompounds. The order of genotoxic activities resulting of the study of Rosenkranz et al. reflects the readiness of the phenols to form phenyldiazoniums rather than their reactivities with $NO^+/NO_2H_2^+$.

Table III lists the activation parameters calculated from the results of experiments to determine the influence of temperature over the range 289–310 K for constant reagent concentrations and acidity (pH 3.5).

Plotting $\Delta H^\#$ against $\Delta S^\#$ (Fig. 5) shows that the reaction with *p*-cresol differs from others in that it fails to exhibit a compensation effect [48], i.e., that the para methyl group which thwarts the tendency of the nitrosonium ion to react at the para position of even activated substrates [49], substantially alters the thermodynamic balance of the formation of the transition state. The contiguity of the O═ and H⟨ N = O groups in the intermediate formed in the *p*-cresol reaction (Scheme II), suggests that this effect of the para methyl group may be due to the rate of proton transfer, being slowed by the formation of an intramolecular hydrogen bond.

Table III Activation Parameters for the Nitrosation of Phenol and Related Compounds. Temperature Range 289–310 K and pH = 3.50

Substrate	$\Delta H^\#$, kJ mol^{-1}	$-\Delta S^\#$, J mol^{-1} K^{-1}
Phenol	43 ± 1	132 ± 7
o-Cresol	31 ± 1	157 ± 12
m-Cresol	32 ± 1	156 ± 12
p-Cresol	35 ± 1	169 ± 16

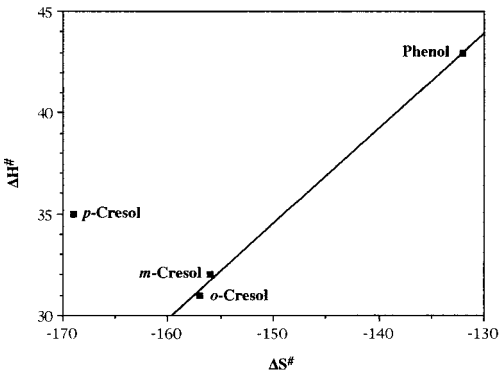


Figure 5 Isokinetic effect for nitrosation reaction of phenol and cresols.

CONCLUSION

The kinetics of the reactions between sodium nitrite and phenol or *m*-, *o*-, or *p*-cresol in potassium hydrogen phthalate buffers of pH 2.5−5.7 shows: (i) At pH $>$ 3 the dominant reaction is C-nitrosation through a mechanism that appears to consist of a diffusion-controlled attack on the nitrosatable substrate by $NO^+/NO_2H_2^+$ ions followed by a slow proton transfer step. The latter step is supported by the observation of basic catalysis by the buffer (which did not form alternative nitrosating agents in the form of nitrosyl compounds); (ii) The observed order of substrate reactivities (*o*-cresol $\approx$ *m*-cresol $>$ phenol $\gg$ *p*-cresol) is explained by the hyperconjugative effect of the methyl group in *o*- and *m*-cresol, and by its blocking the para position in *p*-cresol; (iii) Analysis of the plot $\Delta H^{\#}/\Delta S^{\#}$ shows that the reaction with *p*-cresol differs from those with *o*- and *m*-cresol as regards the formation and decomposition of the transition state; and (iv) The genotoxicity of nitrosatable phenols and their derivatives follows a different order from that of these substances in their reactivities with $NO^+/NO_2H_2^+$.

The authors thank the Commission of the European Communities for supporting the research reported in this article as part of a project on the nitrosatable molecules present in foodstuffs (Contract No. 93CVVF1-610-0). Thanks are also due to the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for its support of research on precursor mechanisms of carcinogenesis by *N*-nitroso compounds (Project No. ALI389/90), and to the Spanish Ministerio de Educación y Ciencia and the Junta de Andalucía for financial assistance to S. González-Mancebo and M. P. Fernández-Liencres, respectively. M. P. Fernández-Liencres also thank J. Casado for facilities afforded during her stay at the University of Salamanca.

BIBLIOGRAPHY

1. B. C. Challis, R. J. Higgins, and A. J. Lawson, *J. Chem. Soc., Perkin Trans. 2,* 1831 (1972).
2. M. Colonna, L. Greci, and M. Poloni, *J. Chem. Soc., Perkin Trans. 2,* 165 (1984).
3. K. Schofield, *Aromatic Nitration,* Cambridge University Press, Cambridge, 1980.
4. H. Bartsch, H. Ohshima, and B. Pignatelli, *Mutat. Res.,* **202,** 307 (1988).
5. H. Ohshima, M. Friesen, C. Malaveille, I. Brovet, A. Haytefeville, and H. Bartsch, *Food Chem. Toxicol.,* **27,** 193 (1989).
6. K. Kikugawa, T. Kato, and K. Kojima, *Mutat. Res.,* **268,** 65 (1992).
7. T. Kato, K. Kojima, K. Hiramoto, and K. Kikugawa, *Mutat. Res.,* **268,** 105 (1992).
8. B. Blackwell and L. A. Mabbitt, *Lancet,* **i,** 938 (1965).
9. S. Yamamoto, S. Wakabayashi, and M. Makita, *J. Agr. Food. Chem.,* **28,** 790 (1980).
10. T. A. Smith, *Food Chem.,* **6,** 169 (1981).
11. K. Wakabayashi, M. Ochiai, H. Saito, M. Tsuda, Y. Suwa, M. Nagao, and T. Sugimura, *Proc. Nat. Acad. Sci. USA,* **80,** 2912 (1983).
12. M. Ochiai, K. Wakabayashi, M. Nagao, and T. Sugimura, *Japan. J. Cancer Res. (Gann),* **75,** 1 (1984).
13. K. Kikugawa, T. Kato, and Y. Takeda, *Mutat. Res.,* **177,** 35 (1987).
14. L. Toth and K. Potthast, *Chemical Aspects of the Smoking of Meat and Meat Products,* C. O. Chichester, E. M. Mrak, and B. S. Schweigert, Eds., in *Adv. Food Res.,* Academic Press, Orlando, 1984, Vol. 29.
15. R. N. Loeppky, Y. T. Bao, J. Bae, L. Yu, and G. Shevlin, *Nitrosamine and Related N-Nitroso Compounds. Chemistry and Biochemistry,* R. N. Loeppky and C. J. Michejda, Eds., *ACS Symposium Series No. 553,* American Chemical Society, Washington, DC, 1994.
16. M. C. Archer, *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer,* I. K. O'Neill, R. C. von Borstel, C. T. Miller, J. Long, and H. Bartsch, Eds., *IARC Sci. Publ. No. 57,* International Agency for Research on Cancer, Lyon, 1984.
17. (a) P. Mende, C.-D. Wacker, R. Preussmann, and B. Spiegelhalder, *Food Cosmet. Toxicol.,* **31,** (1993); (b) R. Davies, R. C. Massey, and D. J. McWeeney, *Food Chem.,* **6,** 115 (1980); (c) E. A. Walker, B. Pignatelli, and M. Castegnaro, *J. Agr. Food. Chem.,* **27,** 393 (1979); (d) T. Kurechi, K. Kikugawa, and T. Kato, *Chem. Pharm. Bull.,* **27,** 2442 (1979); (e) B. C. Challis and C. D. Bartlet, *Nature,* **254,** 532 (1975).
18. D. L. H. Williams, *Nitrosation,* Cambridge University Press, Cambridge, 1988.
19. B. C. Challis and A. J. Lawson, *J. Chem. Soc. (B),* 770 (1971).
20. B. C. Challis and R. J. Higgins, *J. Chem. Soc., Perkin Trans. 2,* 2365 (1972).
21. B. C. Challis and R. J. Higgins, *J. Chem. Soc., Perkin Trans. 2,* 1597 (1973).

22. U. Al-Obaidi and R. B. Moodie, *J. Chem. Soc., Perkin Trans. 2,* 467 (1985).
23. B. D. Beake, J. Constantine, and R. B. Moodie, *J. Chem. Soc., Perkin Trans. 2,* 1653 (1992).
24. B. D. Beake, J. Constantine, and R. B. Moodie, *J. Chem. Soc., Perkin Trans. 2,* 335 (1994).
25. K. Kikugawa and T. Kato, *Food Chem. Toxicol.,* **26,** 209 (1988).
26. T. Ohta, H. Oribe, T. Kameyama, H. Goto, and S. Takitani, *Mutat. Res.,* **209,** 95 (1988).
27. K. Kikugawa, T. Kato, and Y. Takeda, *Chem. Pharm. Bull.,* **37,** 1600 (1989).
28. M. P. Fernández-Liencres, F. Carazo, M. C. Cabeza, B. Quintero, J. Thomas, and J. M. Álvarez, *J. Chem. Soc., Perkin Trans. 2,* 2265 (1993).
29. A. Castro, E. Iglesias, J. R. Leis, M. Mosquera, and E. Peña, *Bull. Soc. Chim. Fr.,* 83 (1987).
30. M. Pires, M. J. Rossi, and D. S. Ross, *Int. J. Chem. Kinet.,* **26,** 1207 (1994).
31. J. Tummavuori and P. Lumme, *Acta Chem. Scand.,* **22,** 2003 (1968).
32. A. I. Biggs and R. A. Robinson, *J. Chem. Soc.,* 388 (1961).
33. K. M. Ibne-Rasa, *J. Am. Chem. Soc.,* **84,** 4962 (1962).
34. S. M. N. Y. F. Oh and L. H. Williams, *J. Chem. Soc., Perkin Trans. 2,* 685 (1991).
35. M. J. Crookes and D. L. H. Williams, *J. Chem. Soc., Perkin Trans. 2,* 1339 (1988).
36. J. Casado, A. Castro, M. Mosquera, M. F. Rodríguez Prieto, and J. Vázquez Tato, *Monatsh. Chem.,* **115,** 669 (1984).
37. S. Senent, J. Casado, and A. de Diego, *Anal. Real Soc. Españ. Fís. Quím.,* **64,** 219 (1968).
38. J. Casado, A. Castro, and M. A. López Quintela, *Monatsch. Chem.,* **112,** 1221 (1981).
39. J. Casado, A. Castro, M. A. López Quintela, and J. Vázquez Tato, *Z. Phys. Chem.,* **127,** 179 (1981).
40. T. A. Turney and G. A. Wright, *J. Chem. Soc.,* 2415 (1958).
41. G. González Alatorre, *Ph. D. Thesis* (Sp), Universidad de Salamanca, 1994, p. 30.
42. J. Casado, A. Castro, M. A. López Quintela, and M. F. Rodríguez Prieto, *Z. Phys. Chem.,* **118,** 43 (1979).
43. J. Casado, A. Castro, J. R. Leis, M. Mosquera, and M. E. Peña, *J. Chem. Soc., Perkin Trans. 2,* 1859 (1985).
44. R. Gil, J. Casado, and C. Izquierdo, *Int. J. Chem. Kinet.,* **26,** 1167 (1994).
45. D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control,* Chapman and Hall, London, 1974.
46. J. March, *Adv. Organic Chem., Reactions, Mechanisms, and Structure,* Wiley, New York, 1992, Chap. 11.
47. H. S. Rosenkranz, G. Klopman, H. Ohshima, and H. Bartsch, *Mutat. Res.,* **230,** 9 (1990).
48. S. Senent, *Cinética Química,* UNED, Madrid, 1986, Chap. 13.
49. E. Bosch and J. K. Kochi, *J. Org. Chem.,* **59,** 5573 (1994).