Sodium Nitrite Reactivity. Part 3.[1] Reaction with Levamisole

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The reaction of aqueous sodium nitrite with levamisole has been studied and the products obtained have been identified by spectroscopic methods, in particular natural abundance ^{13}C and ^{15}N n.m.r. spectroscopy. (*E*)-1-Nitroso-2-oxo-3-(2-nitrosothioethyl)-5-phenylimidazolidine (**3**) is initially formed and this then dimerizes to the disulphide compound by loss of the nitrosyl group on the sulphur atom. Derivatives of levamisole have also been prepared for the determination of the reaction mechanism and identification of the products.

The laevorotatory isomer of tetramisole, levamisole. (**1**), is a therapeutic agent, used as an anthelmintic in animal[2,3] and human subjects.[3,4] Its tolerance or highest admissible concentration as a residue in food from animal origin is fixed at 0.1 p.p.m. In recent years it has been used clinically for potentiating the human immunatory system,[3,4] in spite of secondary effects.[4,5] This compound is also a potent alkaline phosphatase inhibitor used in biochemical studies[6–9]. In spite of this interest however there has been little work on the reactivity of levamisole towards nucleophilic reagents.[10] Although the levamisole base (**2**) is insoluble in water its hydrochloride (**1**), a cyclic thiouronium salt, is very soluble (21%).[2] The formation of nitrosamines, known to be powerful carcinogenic agents, was detected during a study of the reactivity of various iminium salts toward sodium nitrite. It was, therefore, of interest to investigate the reaction of $NaNO_2$, a common food additive, with this cyclic iminium salt whose therapeutic use often necessitates the use of arbitarily determined doses.[4] The reaction between levamisole (**1**) and sodium nitrite was studied in aqueous medium (pH ≃ 5), comparable with biological conditions. The products (**3**) and (**4**) were identified by use of ^1H, ^{13}C, and ^{15}N n.m.r. spectroscopy and by comparison with related compounds. A reaction mechanism is proposed.

Experimental

Materials.—Levamisole hydrochloride (**1**) was purchased from Aldrich; $\delta_H(D_2O$, 60 MHz) 3.60 (1 H, dd, *J* 8.5 and 10 Hz), 3.83 (4 H, m), 4.22 (1 H, t, *J* 10 Hz), and 5.67 (1 H, dd, *J* 8.5 and 10 Hz); $\delta_C(H_2O)$ 37.7 (CH_2S), 49.1 (CH_2N), 56.0 (CH_2N), 68.4 (CH), 127.6, 130.3, 130.5, and 139.4 (C aromatic), and 177.7 (C-2); $\delta_N(H_2O$, p.p.m./CH_3NO_2) −255.5 (N-1) and −266.2 (N-2).

Levamisole (**1**) (1.7 g, 7.0 mmol) was added at room temperature to a solution of sodium nitrite (1 g, 14.5 mmol) in water (8 ml) and the pH of the solution was adjusted with aqueous HCl. The precipitate of (**3**) was filtered off and either analysed (i.r., mass spectrum) or dissolved in chloroform. The red solution in chloroform became gradually yellow and after 1 or 2 days, the white precipitate obtained (**4**) was separated and analysed: (**3**), $\delta_H(CDCl_3$, 250 MHz) 3.33 (1 H, dd, *J* 3.3 and 9 Hz), 3.5—4.1 (5 H, m), and 5.27 (1 H, dd, *J* 3.3 and 9 Hz); $\delta_C(CHCl_3)$ 31.7 (CH_2S), 44.0 (CH_2N), 52.3 (CH_2N), 55.0 (CH), 126.5, 129.8, 130.3, and 138.2 (C aromatic), and 154.1 (C-2); *m/z* 280 (M^+), 250, 204, 189, 175, and 132.

(**4**), $\delta_H(Me_2SO$, 250 MHz) 3.1 (2 H, t, *J* 6, 5 Hz), 3.4 (1 H, dd, *J* 3.3 and 10 Hz), 3.6 (1 H, dt, *J* 6.5 and 14 Hz), 3.9 (1 H, dt, *J* 6.5 and 14 Hz), 4.0 (1 H, dd, *J* 9 and 10 Hz), and 5.43 (1 H, dd, *J* 3.3 and 9 Hz); $\delta_C(CHCl_3)$ 36.4 (CH_2S), 43.6 (CH_2N), 52.0 (CH_2N), 55.1 (CH), 126.8, 129.7, 130.3 and 138.7 (C aromatic), and 153.9 (C_2); $\delta_N(Me_2SO)$ +184.1 (NO), −110.6 (N-1), and −287.0 (N-2); *m/z* 442, 221, 189, 175, and 132.

Levamisole (**1**) (1 g) was dissolved in water (5 ml) and the pH of the solution was adjusted to 9 by addition of Na_2CO_3. The organic layer was extracted with chloroform, dried, and evaporated. Compound (**2**) so obtained reacted with an equimolor quantity of dimethyl sulphate to give (**6**).

(**2**), $\delta_H(CHCl_3$, 60 MHz) 2.77—3.77 (6 H, m) and 5.33 (1 H, t, *J* 8.5 Hz); $\delta_C(CHCl_3)$ 35.2 (CH_2S), 50.2 (CH_2N), 59.4 (CH_2N), 77.8 (CH), 127.6, 128.3, 129.6 and 143.9 (C aromatic), and 175.5 (C-2); $\delta_N(CHCl_3)$ −172.0 (N-1) and −269.0 (N-2).

(**6**), $\delta_H(D_2O)$, 60 MHz) 3.0 (3 H, s), 4.0 (3 H, s), 3.7—4.6 (6 H, m), 5.7 (1 H, dd, *J* 8.5 and 10 Hz) (see ref. 10a); $\delta_C(CHCl_3)$ 34.8 (CH_3N), 38.5 (CH_2S), 50.2 (CH_2N), 55.4 ($MeSO_4^-$), 56.0 (CH_2N), 74.1 (CH), 129.0, 130.7, 131.0 and 136.6 (C aromatic), and 178.3 (C_2); $\delta_N(CHCl_3)$ −258.6 (N-1) and −268.0 (N-2).

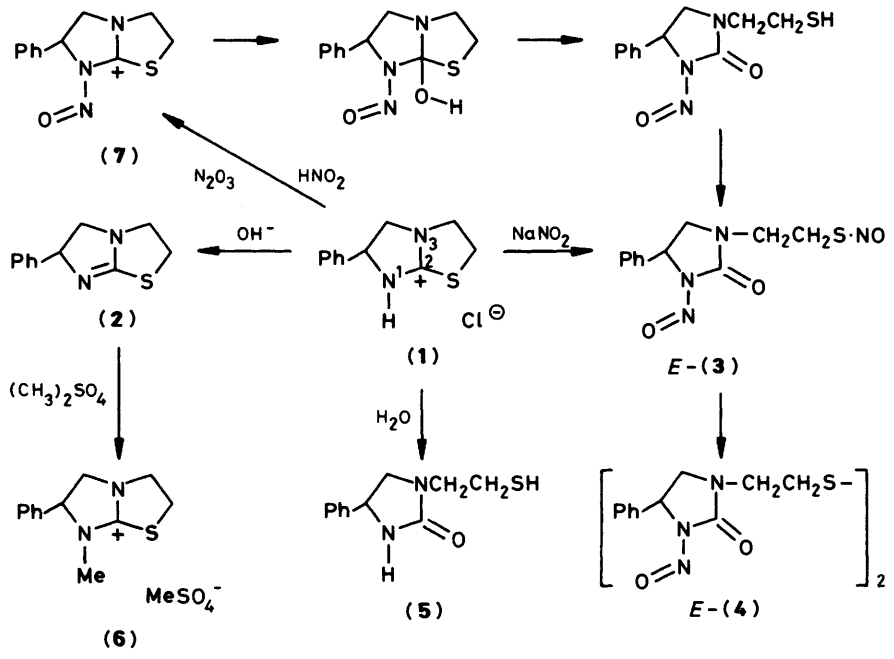
Levamisole (**1**) (0.5 g) dissolved in water (5 ml) was heated (373 K) for 24 days in sealed ampoules. The organic product (**5**) was extracted with chloroform and analysed; $\delta_H(CHCl_3$, 250 MHz) 1.25 (1 H, t), 2.65 (1 H, q), 2.84 (1 H, t), 3.25—3.45 (3 H, m), 3.86 (1 H, t, *J* 8 Hz), and 4.80 (1 H, m) (see refs 10c and 15); $\delta_C(CHCl_3)$ 24.0 (CH_2S), 47.7 (CH_2N), 55.0 (CH_2N), 55.2 (CH), 127.1, 129.4, 130.1, and 142.6 (C aromatic), and 162.4 (C-2).

Spectroscopic Measurements.—^1H Spectra were studied on a Hitachi-Perkin-Elmer R 24 (ν_o = 60 MHz) or a Brüker WM 250 (ν_o = 250 MHz) spectrometer with products in solution in D_2O (**1**) and (**6**), $CHCl_3$ (**2**), (**3**), (**5**), or Me_2SO (**4**). The ^{13}C chemical shifts at 22.635 MHz were measured at 300 K using a Brüker WH-90 spectrometer: SW = 6 000 Hz; AT = 0.679 s; PW = 25 °; solvent: $CHCl_3$ except for (**1**) (H_2O). Chemical shifts are given in p.p.m. from external $SiMe_4$ in C_6D_6. ^{15}N Spectra were recorded with a Brüker WM 250 spectrometer at natural abundance level: SW = 9 000 Hz; AT = 0.9 s; PW = 45°; ν_o = 25.349 MHz; broad band decoupling (2 W); *T* = 298 K. The product (**1**) was dissolved in water (2M, pH = 4.2), (**2**) and (**6**) in $CHCl_3$, and compound (**4**) in Me_2SO; with organic solvents, [Cr(acac)$_3$] (0.08M) was added to the samples. The shifts were referred to an external CD_3NO_2 solution of ^{15}N enriched CH_3NO_2 contained in a coaxial 4 mm tube centred in a 15 mm sample tube.

I.r. spectra were obtained with a Philips-Pye-Unicam SP 1 100 spectrophotometer as KBr pellets and the mass spectra on a Varian MAT 112 instrument.

Results and Discussion

Structural Identification.—At room temperature, Levamisole reacts rapidly with sodium nitrite in an aqueous medium (pH = 5); the pink precipitate (**3**) which appears dissolves easily in



$CHCl_3$ and gives a red solution which becomes yellow within a few hours and finally gives a white solid (**4**) (Scheme).

The ^1H and ^{13}C n.m.r. spectra of the two products (**3**) and (**4**) are identical except for the ^{13}C chemical shift of the CH_2 group close to the sulphur atom: 31.7 and 36.4 p.p.m. respectively. Comparison with compound (**5**) (δ_C = 24.0 p.p.m.) formed by hydrolysis of levamisole and having a thiol function shows that compounds (**3**) and (**4**) lack this function. Moreover, the $\delta_C(CH_2S)$ of the products (**5**), (**3**), and (**4**) vary in the same order as those of *N*-acetylcysteine (26.8 p.p.m.), *S*-nitroso-*N*-acetylcysteine (34.1 p.p.m.), and *N*-acetylcystine (41.5 p.p.m.). These results suggest that compound (**3**) contains a nitrosothio function, capable of dimerizing to form the disulphide (**4**);[12] they also explain the similarity of the ^1H and ^{13}C spectra of these two compounds.

The nitrosothio function in solution is rather unstable and the ^{15}N spectrum of (**3**) was not obtained. However, the ^{15}N chemical shifts of compound (**4**) show the presence of nitrosamine and amide functions.[13] The δ_C signal (154 p.p.m.) for the carbonyl group of compounds (**3**) and (**4**) is in good agreement with a nitrososurea framework; the $\Delta\delta_C$ difference observed between (**3**) [or (**4**)] and (**5**) (δ_C = 162.4 p.p.m.) is similar to that reported for trimethylnitrosourea[14] and trimethylurea (159.9 p.p.m.).

I.r. spectroscopy supports the proposed structures. The carbonyl absorption band at 1 760 cm^{-1} for (**3**) and (**4**) undergoes a hypsochromic shift (50—70 cm^{-1}) as compared with the urea (**5**);[10c,15] a similar effect has been already mentioned between linear nitrosoureas and ureas themselves.[16] Moreover, relatively intense bands at 1 480, 1 435, 1 315, and 1 180 cm^{-1} are in accordance with a nitrosamine group.[16] The i.r. spectra of compounds (**3**) and (**4**) are similar except for the appearance of a broad band in the region at 1 500—1 530 cm^{-1} for (**3**); this band may be assigned to the nitrosothio function of (**3**).[12]

The mass spectrum of compound (**4**) shows a prominent ion of mass 221 and an ion of mass 442: this result is in agreement with a dimeric structure. For compound (**3**), the molecular ion was detected at *m/z* 280; other ions detected in accord with the proposed formula.

The use of these various spectroscopic methods has enabled the structure of the two compounds (**3**) and (**4**) to be unambiguously determined. The n.m.r. spectra of the two products obtained show the presence of only one isomer, although nitrosamines may be subject to *Z*/*E* isomerism with high activation parameters ($\Delta G^{\neq}$) for the rotation about N–N bonds.[17] In such isomers, the carbon atoms in a *cis* position with respect to a nitroso group are at higher fields (δ_C8—10 p.p.m.) than *trans* carbon atoms.[17] Moreover, introduction of a nitroso function into trimethylurea gives rise to a lowfield shift of 1 to 2 p.p.m. for the α carbon *cis* to N=O:[14] this shift would be *ca.* 10 p.p.m. for the *trans* carbon atom. The difference in the ^{13}C chemical shifts observed for the cyclic urea (**5**) (55.2 p.p.m.) and the nitroso compounds (**3**) and (**4**) (55.0 p.p.m.) for the α carbon (CH) is close to 0 and suggests an *E* structure (Scheme). ^1H N.m.r. spectroscopy confirms the *E* configuration: thus, the δ_H values for compounds (**3**), (**4**), and (**5**) are respectively 5.27, 5.43, and 4.80 for the proton located in an α position to N=O. Thus the n.m.r. signals for compounds (**3**) and (**4**) are shifted to a lower field by 0.47 to 0.63 p.p.m. and are similar to those observed for dimethylamine and dimethylnitrosamine in which the protons are deshielded by 0.66 p.p.m. for the *cis* or 1.42 p.p.m. for the *trans* position with respect to the N=O.

Mechanism.—In order to elucidate the mechanism of the reaction being studied, a number of compounds derived from levamisole (**1**) were also investigated studied. In basic medium levamisole gives, by loss of HCl,[4] compound (**2**) and the action of dimethyl sulphate upon the latter produces a new iminium salt (**6**), methylated at N-1; the ^1H n.m.r. parameters of this salt are identical with those reported previously.[10a] ^{15}N Chemical shifts for compounds (**1**), (**2**), and (**6**) are given in the Experimental section and are consistent with their structure. Sodium nitrite reacts neither with the levamisole base (**2**) nor with the imidazothiazolinium salt (**6**).

Amidinium salts are stable towards $NaNO_2$–H_2O in acidic medium at room temperature, although they undergo hydrolysis at higher temperatures (343—363 K).[11] The behaviour of levamisole (**1**) is quite different, in that it reacts rapidly with aqueous sodium nitrite at room temperature but undergoes hydrolysis only under extreme conditions.[15] Clearly, the first step of the reaction is attack by nitrite. The homologous methylated salt (**6**), however is unreactive at C-2 nitrite attack, the positive charge at this position being stabilized by

delocalisation in the presence of two nitrogen atoms and one sulphur atom (*cf.* amidinium salts[11]). The two salts (**1**) and (**6**) differ only in the presence of a methyl group at 1-N on the latter; nitrosamine formation in (**3**) and (**4**) suggests attack in the NH group by the nitrosating reagent N_2O_3 or HNO_2[18] (Scheme). Since in the new iminium salt (**7**), the nitroso function and N-1 are conjugated the lone-pair of the latter is delocalized towards the former; this results in a high value of free enthalpy of activation $\Delta G^{\neq}$ for rotation about the N–N bond.[17] In view of this delocalisation the N-1 lone pair will contribute little to stabilisation of the carbocation. The latter may be considered from an electronic point of view, as a salt derived from a simple thioamide: such thioamidium salts react quickly with aqueous sodium nitrite to form quantitatively the amide function by hydrolysis of the C–S bond.[11] In the case of the intermediate (**7**), this hydrolysis explains the formation of the thio and nitrosourea functions. The thio group is then nitrosated and gives the nitrosothio compound (**3**)[12] which dimerizes to the disulphide compound (**4**).

An alternative reaction mechanism may also be considered in which levamisole (**1**) first undergoes nitrosation at S instead of N. A precedent for this is the rapid *N*-nitrosation of L-methionine and *S*-methylcysteine which has been explained in terms of an initial *S*-nitrosation followed by a rearrangement of the molecule so that a 1,4- or 1,5-shift of the nitroso group can occur.[19,20] A further example is provided by the *N*-nitrosation of thiourea: at low acidity, it has been suggested that the first step of the reaction is preferential S attack,[21] but a ^{15}N n.m.r. study indicates direct nitrosation at the nitrogen atom.[18]

It must be kept in mind that nitrosating species are related to the acidity of the medium. Dilute acid conditions favour unprotonated nitrous acid and N_2O_3. Since these species may be harder acids than the nitrosonium ion, they will favour attack at the harder nitrogen atom.[18] On the other hand, softer acids such as the nitrosonium ion NO^+ or its derivatives, are formed at higher acidity and favour *S*-nitrosation.[19] Such behaviour has been recently discussed in terms of charge and frontier orbitals.[22] Since the reaction of levamisole has been studied in very dilute acid conditions (pH = 5) a *N*-nitrosation would be expected. Nevertheless, an initial *S*-attack cannot be excluded and the *S*-nitroso intermediate could undergo an intramolecular (or intermolecular) *S*-to *N*-rearrangement. This migration would be effective with levamisole (**1**) but not with methylated compound (**6**).

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