Tetrahedron. Vol. 34, pp. 505 to 508. Pergamon Press 1978. Printed in Great Britain

A NEW N-NITROPYRROLE

1,4-DINITRO-2-METHYLPYRROLE, FORMED BY THE REACTION OF SORBIC ACID WITH SODIUM NITRITE

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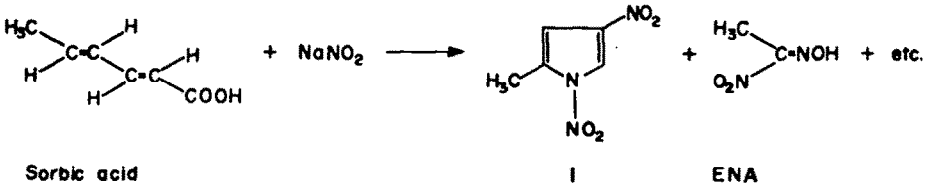
(*Received in Japan* 14 *May* 1977; *Received in the UK for publication* 22 *September* 1977)

Abstract—A new N-nitropyrrole, 1,4-dinitro-2-methylpyrrole, has been isolated and identified as one of the active products of the acidic reaction mixture of sorbic acid and sodium nitrite in the rec-assay and growth inhibition tests on bacteria. The various spectral data that led to the proposed structure, and some of its chemical properties are described.

Kada has previously shown that a strong DNA damaging activity on bacteria is developed when a mixture of sorbic acid and sodium nitrite is heated on a boiling water bath.[1] Ethylnitrolic acid (ENA) is possibly one of the active products in the mixture.[2] When the reaction was carried out without pH control (initial pH 4.3; final pH 6.0), a marked dependence of the processes as well as the yields of the active agents on the pH of the reaction was noted. While the yield of ENA was maximum when the pH was controlled at pH 4.2, the growth inhibitory activity was greatest at a reaction of pH 3.5. The present paper is concerned with the isolation of a new product, 1,4-dinitro-2-methylpyrrole, from the reaction and its structural elucidation mainly based upon various spectroscopic studies.



Sorbic acid I ENA

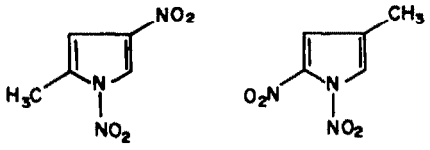
reasonably interpreted as a combination of the NO_2, NO, 3.5, (Experimental) yielded after purification by chromatography a crystalline product, (1) m.p. 136–137°.

Elemental analysis of 1 was consistent with the formula $C_5H_5O_4N_3$, and high resolution mass spectrum of 1 gave a molecular ion peak at *m/e* 171.0284 corresponding to $C_5H_5O_4N_3$ (*m/e* 171.0279).

The ^1H NMR spectrum of 1 in $CDCl_3$ showed an AB-type signal with two aromatic protons at 7.91 (d, 1H, $J = 2.93$ Hz) and 8.88 (d, 1H, $J = 2.93$ Hz) and one Me group absorption peak at 2.65 (s. 3H). Furthermore, the ^{13}C FT NMR spectrum of 1 in $CDCl_3$ exhibited four aromatic ^{13}C signals, two doublets and two singlets in the off-resonance decoupled spectrum, in the region of 113–141 and one Me signal at 19.1. 1 was positive to the pine splinter test giving a red color indicating the presence of a pyrrole ring, and the NMR signals are consistent with those known for pyrrole derivatives.[3,4] If 1 is assumed to be a Me-substituted pyrrole, the facts that no appreciable change was observed in the ^1H NMR signals by the addition of D_2O or a trace of piperidine[5] to the $CDCl_3$ solution of 1 at room temperature, and that the absorption band around 3400–3500 cm^{-1} is absent in the IR spectrum, suggest the absence of a pyrrole N–H group. Moreover, the fact that 1 was positive to Ehrlich's reagent[6] but became negative when treated with diazomethane to give 3 seems to mean at one α-position of the pyrrole ring of 1 is free or potentially free. Two signals of aromatic methine groups at δ_C 124.3; δ_H 8.88 and δ_C 113.2; δ_H 7.91 (assigned by the ^1H single-frequency off resonance decoupled spectra), are very different from each other in the chemical shifts, especially in ^{13}C NMR, and are assigned to two methine groups at α and β-positions, respectively. These data reasonably lead to the assumption that 1 is an N,α,β-trisubstituted pyrrole containing one Me group.

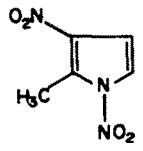
The unassigned part of the formula, N_2O_4, may be resonably interpreted as a combination of the NO_2, NO, ONO_2, and ONO groups. However, ONO and ONO_2 groups can be eliminated based on the absence of characteristic IR bands. The nitroso group must also be absent because 1 did not absorb at 760–770 nm, which is characteristic for aromatic nitroso compounds, and also it was negative to the color reaction with diphenylamine–$PdCl_3$ for detection of nitrosamines. These results indicate the absence of the functional group NO in the molecule of 1.

On the other hand, IR absorption bands situated at 1525 and 1580 cm^{-1} ($CHCl_3$) seem to be attributable to ν_{as} C–NO_2 and ν_{as} N–NO_2, respectively. Since the ^1H chemical shift of the Me proton of N-methylpyrroles is usually larger than 3.5, while that of 1 was 2.65, 1 is probably a N-nitro rather than N–Me pyrrole. Consequently, the structure of 1 was presumed to be one of the following.

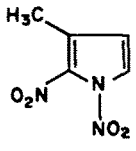


I

II



III

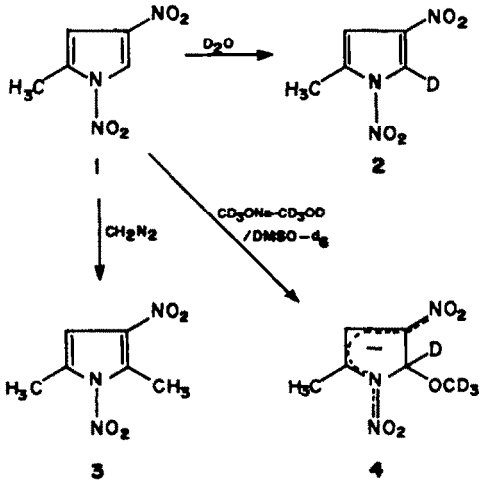


IV

Treatment of 1 with diazomethane gave the methylated derivative 3 as yellow needles, m.p. 160–161°. The ^1H and ^{13}C NMR spectra of 3 were devoid of signals corresponding to the α-methine group, instead showing new signals due to a Me group (δ_C 15.7; δ_C 2.83), an indication that the α-methine group of the pyrrole was methylated during the treatment. The ^1H chemical shift of the Me signal of 3 was downfield with respect to that of the original, but the opposite was true for the ^{13}C chemical shift. The original Me group in the undecoupled ^{13}C NMR spectrum of 3 showed long-range coupling (d, J = 3.1 Hz) identical with that of 1 but the new one showed no long-range coupling. It was thus concluded that structure I is the most reasonable one for 1.

Based on the structure 3, it seems likely that appearance of the ^{13}C chemical shift of the new Me group at a higher field than that of the original might have been caused by a steric compression in the structure. Moreover the two quarternary ring carbons thus formed at α-carbons of the pyrrole ring result in the same value for the chemical shift of the ^{13}C NMR signal.

Consequently, the ^1H and ^{13}C NMR spectra of 1 and 3 were assigned as shown in Table 1.



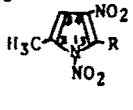
1 2 3 4

The ^1H NMR signal of the α-proton of 1 in $CDCl_3$ or DMSO-d_6 showed no significant change with the addition of D_2O, but it disappeared completely when a pyridine-d_6 solution of 1 was left for 2 days in an ice box after the addition of D_2O. Moreover, when 1 was dissolved in D_2O by heating, the signal that appeared at 9.36 (Hα) as doublet in the initial stage disappeared almost completely

Table 1. ^1H and ^{13}C NMR data[a] of 1 and 3

Position No.[b]	1		3	
	δ_H	δ_C	δ_H	δ_C
2		140.6 (seemingly broad q 6.7)		138.4 (–)[c]
3	7.91 (d 2.93)	113.2 (d 180.7, d 4.3, q 4.3)	7.74 (s)	113.1 (d 177.0, q 4.3)
4		136.2 (seemingly broad d 3.7)		137.4 (–)[c]
5	8.88 (d 2.93)	124.3 (d 200.2, d 5.5)		138.4 (–)[c]
2-Me	2.65 (s)	19.1 (q 131.8, d 3.1)	2.61 (s)	18.6 (q 132.4, d 3.1)
5-Me			2.83 (s)	15.7 (q 134.3)

[a] Solvent : $CDCl_3$ ($J_{^{13}CH}$ of 1 was measured in DMSO-d_6), δ : ppm from TMS , The figures in parentheses relate to coupling constant in Hz.

[b]  1 : R=H, 3 : R=CH_3

[c] No assignment because of the overlap of 2,4, and 5 signals

after 2 hr, while the one that appeared at 8.36 ($H\beta$) as a mixture of singlet and doublet collapsed into a singlet. No significant change was observed in D_2O acidified with DCl. That a nitropyrrole easily undergoes a base-catalyzed hydrogen exchange at the α-position has been reported,[7] but the present case is of interest because of the highly reactive α-proton proved exchangeable without addition of a base catalyst. This also explains the fact that the α-position of 1 was easily methylated by treatment with CH_2N_2.

Addition of an equivalent amount of methanolic sodium methoxide to a DMSO-d_6 solution of 1 readily colored brown, along with the significant changes in the ^1H NMR spectrum. Immediately after the addition, all proton signals due to 1 disappeared and two singlets at 2.00 and 6.93 (3H; 1H), which may be assigned to Meisenheimer-type adduct 4,[7] appeared together with other unidentified signals probably related to decomposition products. Prolonged reaction seemed to lead to decomposition of 4 along with complete disappearance of the two singlets. No hydrogen exchange reaction was observed, probably being obscured by the formation of Meisenheimer-type adduct. Failure to observe an α-proton signal of the adduct might indicate a sufficiently rapid hydrogen exchange reaction at the α-position along with the addition reaction.

Each of the mass spectra of 1 (M^+ 171), 2 (M^+ 172) and 3 (M^+ 185) exhibited M-16 (M–O) and M-30 (M–NO) ion peaks indicating the presence of NO_2 groups, though there was no peak at M-46 (M–NO_2). 1 gave a base peak at *m/e* 39 (probably cyclopropenyl ion), and this peak was also very strong in the mass spectrum of 3. The formation of cyclopropenyl ion as the base peak of pyrroles has well been known[8] but the formation pathway may not be the same for unusually substituted pyrroles such as 1 and 3.

The addition of an alkali markedly changed the UV spectrum of 1. At pH 9.9 (D_2O + 1 N NaOH) the absorption maxima at 229 and 270 nm underwent hypochromic change, while those at 328 and 368 nm suffered hyperchromic red shift and hyperchromic blue shift, respectively. Since these changes were not reversed by neutralization with HCl, the addition of NaOH must have caused rapid decomposition of 1 in an irreversible manner. In the case of the methylated compound 3, no significant change in the UV spectrum was observed with alkali addition. All absorption maxima of 1 and 3 showed a blue shift in polar solvents. The UV spectrum of 3 in 2 M H_2SO_4 remained unchanged even after the compound was left at 60° for 24 hr. Extraction from this solution recovered unaltered 3 which showed no shift of absorption by alkali. These facts may also evidence the absence of ONO and ONO_2 groups in the molecules of 3 and 1.

In conclusion, various spectral data reasonably lead to identification of the newly isolated product 1 as a new N-nitropyrrole, 1,4 - dinitro - 2 - methylpyrrole. The machanism of formation of 1 is interesting but not much can be said about it at this stage except that the pyrrole ring skeleton is probably derived from sorbic acid by nitration or nitrosation followed by decarboxylation.

EXPERIMENTAL

All materials and other reagents used were of guaranteed grade.

M.p. were determined by a Yanagimoto micro m.p. apparatus and are uncorrected. UV spectra were recorded on a Hitachi EPS-3T recording spectrometer. IR spectra were recorded on a JASCO Model IR-G grating spectrophotometer. ^1H NMR spectra were mainly obtained with a JNM-MH-100 and partly on JNM-FX 60 and JNM-FX 100 spectrometers. ^{13}C NMR spectra were recorded with JNM-FX 60 and JNM-FX 100 spectrometers. The chemical shifts (δ) were calculated on the basis of TMS as an internal standard unless otherwise noted. Mass spectra were recorded on JMS-D100 and JMS-01SG-2 spectrometers operated at 75 eV. PH control of reaction mixture was done by use of a Radiometer Titrator TTT-2 pH stat with dil. H_2SO_4. Fluorescent silica gel (Wakogel B-5FM) was used for TLC and spots on TLC plates were usually detected by irradiation with a PAN-UV-Lamp PUV-1.

1,4-Dinitro-3-methylpyrrole 1. A soln of $NaNO_2$ (82.8 g) was added to a partially suspended soln of sorbic acid (16.8 g) in distilled water (1.5 l). Keeping the pH of the mixture constant at 3.5 with dil. H_2SO_4, the mixture was stirred at 60° for 2 hr. The mixture was extracted with three 500 ml portions of CH_2Cl_2. The combined extracts were washed with water, dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give 3.5 g of residue. The residue was chromatographed on a silica gel column with $CHCl_3$, and fractions were monitored by TLC ($CHCl_3$–MeOH, 97:3). The fractions that gave a single yellow spot of 1 were collected and concentrated *in vacuo* to give approximately 1 g of 1, yellow needles (from $CHCl_3$), m.p. 136–137° (Found: C, 35.62; H, 2.97; N, 24.86. Calc. for $C_5H_5N_3O_4$: C, 35.09; H, 2.95; N, 24.56%): UV λ_{max} (H_2O) 210 nm (log ϵ 4.03), 229 (4.20), 270 (4.10), 329 (3.91), 386 (3.23); λ_{max} (H_2O + 1N NaOH, pH 9.9, about 20 min after the addition) 299 nm (log ϵ 4.08), 345 (4.01); λ_{max} (EtOH) 212 nm (log ϵ 4.02), 234 (4.19), 275 (4.11), 336 (3.88), 398 (3.28); λ_{max} (n-hexane) 237 nm, 282, 288 (sh), 340 (sh), 350, 396 (sh), 409, 428; IR (KBr) 3080 (pyrrole ring νCH), 3050 (pyrrole ring νCH), 1590 (N-NO_2 ν_{as} NO_2), 1550 (sh) (C-NO_2 ν_{as} NO_2), 1530 (C-NO_2 ν_{as} NO_2), 1470 (δ_{as} CH_3), 1420, 1389, 1380, 1345 (ν_s NO_2), 1122, 1098, 1030, 965, 910, 800, 750, 565 cm^{-1}; IR ($CHCl_3$) 3100 (pyrrole ring νCH), 1603 (pyrrole ring νC = C), 1580 (N-NO_2 ν_{as} NO_2), 1540 (sh) (C-NO_2 ν_{as} NO_2), 1525 (C-NO_2 ν_{as} NO_2), 1473 (δ_{as} CH_3), 1425, 1386, 1342 (ν_s NO_2), 1121, 1093, 968, 865, cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^1H NMR (DMSO-d_6) 2.49 (s, 3H), 8.36 (d, 1H, J = 2.44 Hz), 9.38 (d, 1H, J = 2.44 Hz); ^1H NMR (pyridine-d_5) 2.46 (s, 3H), 8.14 d, 1H, J = 2.9 Hz), 9.67 (d, 1H, J = 2.9 Hz); ^1H NMR (D_2O + DCl, δ from DSS)2.61 (s,3H), 8.33 (d, 1H, J = 2.93), 9.34 (d, 1H, J = 2.93); ^{13}C NMR ($CDCl_3$) see Table 1; ^{13}C NMR (DMSO-d_6) 18.0 (off-resonance q), 114.0 (d), 125.2 (d), 136.7 (s), 140.9 (s); MS *m/e* (relative intensity) 27 (22), 28 (CH_2N^+, 64), 29 (5), 30 (NO^+, 92), 32 (14), 37 (7), 38 (22), 39 ($C_3H_3^+$, 100), 40 (16), 41 ($C_2H_3N^+$, 20), 42 (16), 43 (39), 44 (10), 50 (8), 51 (11), 52 (12), 53 (9), 54 (6), 55 (11), 57 (13), 62 (13), 62 (9), 63 (24), 64 (18), 65 (45), 66 (11), 67 (6), 68 (5), 69 (7), 71 (7), 79 (5), 82 (8), 83 (5), 85 (5), 94 (6), 124 (M^+–HNO_2, 5), 141 (M^+–NO, 6), 155 (M^+–O, 6), 171 (M^+, 60). MW; Calc. for $C_5H_5N_3O_4$: 171.0280. Found (high-resolution MS): 171.0284. Both the pine splinter test by the use of match-wood moistened with conc. HCl and color reaction with Ehrlich's reagent were positive. This compound was negative to the diphenylamine–palladium chloride reagent for detection of nitrosamines.

1,4-Dinitro-3-methylpyrrole-5-d 2. 1 was dissolved in D_2O with heating, and allowed to stand overnight. After a hydrogen exchange had been confirmed from the ^1H NMR spectrum, the soln was extracted with $CHCl_3$, dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give 2: ^1H NMR ($CDCl_3$) 2.63 (t, 3H), 7.94 (s, 1H); ^1H NMR (D_2O) 2.62 (t, 3H), 8.36 (s, 1H); MS *m/e* (relative intensity) 27 (19), 28 (CH_2N^+, 45), 29 (6), 30 (NO^+, 100), 32 (8), 37 (7), 38 (13), 39 ($C_3H_3^+$, 72), 40 ($C_3DH_2^+$, 72), 41 ($C_2H_3N^+$, 19), 42 (11), 43 (49), 44 (15), 50 (11), 51 (10), 52 (13), 53 (12), 54 (17), 55 (9), 56 (5), 57 (9), 62 (7), 63 (16), 64 (29), 65 (24), 66 (63), 67 (11), 68 (7), 69 (5), 72 (9), 80 (6), 82 (9), 94 (7), 95 (5), 110 (6), 124 (M^+–DNO_2, 5), 142 (M^+–NO, 16), 156 (M^+–O, 8), 172 (M^+, 85).

1,3-Dinitro-2, 5-dimethylpyrrole 3. A soln of diazomethane in ethyl ether (200 ml) and MeOH (1 ml) was added to a soln of 1 (0.6 g) in $CHCl_3$ (200 ml). The soln was allowed to stand for 11 hr at 0° and then for 11 hr at room temp. TLC of the mixture showed two main spots, R_f-values 1.1 and 0.58 (relative to that

of 1, with $CHCl_3$–MeOH 19:1), which showed up purple and reddish brown under UV light (PAN-UV-Lamp), and yellow and colorless under visible light, respectively. There was no spot detected for 1. The mixture was evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel column. The yellow main product 3 (R_{f_1} 1.1) and another product (R_{f_1} 0.58) were eluted with $CHCl_3$ and $CHCl_3$–MeOH (98:2), respectively. 3 (90 mg), m.p. 161–162°, was obtained as yellow needles, (recrystallized from $CHCl_3$–n-hexane). (Found: C, 38.68; H, 3.85; N, 22.74. Calc. for $C_6H_7O_4N_3$: C, 38.92; H, 3.81; N, 22.70%): UV λ_{max} (H_2O) 217 nm (sh) (log ϵ 4.07), 234 (4.24), 268 (4.03), 325 (3.82), 378 (sh) (3.71); λ_{max} (EtOH) 220 nm (sh) (log ϵ 4.05) 237 (4.24), 275 (4.05), 332 (3.80), 390 (3.21); λ_{max} (n-hexane) 222 nm (sh), 240, 267 (sh), 281, 286 (sh), 317 (sh), 337 (sh), 346, 410; IR (KBr) 3080 (pyrrole ring νCH), 1582 (N-NO_2 ν_{as} NO_2), 1530 (C-NO_2 ν_{as} NO_2), 1470 (δ_{as} CH_3), 1457, 1429, 1405, 1375, 1355, 1340, 1312, 1154, 1057, 1039, 885, 860, 778, 760, 730, 561 cm^{-1}; IR ($CHCl_3$) 3090 (pyrrole ring νCH), 1582 (N-NO_2 ν_{as} NO_2) 1534 (C-NO_2 ν_{as} NO_2), 1485 (δ_{as} CH_3), 1458, 1420, 1389, 1380, 1355, 1339, 1315, 1150, 1050, 1038, 870, 840 cm^{-1}; ^1H NMR ($CDCl_3$) see Table 1; ^1H NMR (D_2O) 2.53 (s, 3H), 2.72 (s, 3H), 8.01 (s, 1H); ^{13}C NMR ($CDCl_3$) see Table 1; MS *m/e* (relative intensity), 27 (47), 28 (7), 29 (6), 30 (NO^+, 81), 37 (8), 38 (11), 39 ($C_3H_3^+$, 63), 40 (16), 41 (25), 42 (25), 43 (39), 50 (36), 51 (80), 52 (50), 53 (52), 54 (10), 55 (14), 57 (13), 63 (15), 64 (7), 65 (8), 66 (42), 67 (22), 68 (11), 69 (7), 71 (6), 74 (8), 75 (5), 76 (5), 77 (100), 78 (36), 79 (12), 80 (6), 81 (6), 82 (11), 83 (6), 84 (6), 85 (8), 86 (5), 92 (6), 93 (8), 108 (8), 109 (6), 125 (15), 138 (M^+–HNO_2, 8), 154 (5), 155 (M^+–NO, 17), 169 (M^+–O, 3), 185 (m^+, 51).

The pine splinter test on 3 was positive (red) and Ehrlich's test negative. The compound was treated with 2 M H_2SO_4 at 65° for 2 days. The soln was extracted with CH_2Cl_2, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The extract gave only one spot of 3 on TLC. The UV spectrum (H_2O) of the extract was identical to that of 3 and was not altered by the addition of NaOH.

Meisenheimer-type adduct of 1*A*. An equivalent amount of 3 M methanolic NaOMe was added to a 0.33 M soln of 1 in DMSO-d_6. The ^1H NMR spectrum was recorded at regular intervals after the addition. ^1H NMR data suggested the formation of a Meisenheimer-type adduct: ^1H NMR (immediately after the addition) 1.5–2.3 (4H, excluded 3H of s at 2.00), 2.00 (s, 3H), 3.6–4.3 (broad, Ca. 11H), 6.93 (s, 1H).

Acknowledgements—This work was supported by a grant from Nissan Science Foundation. The authors thank Mr. H. Ito for his excellent technical assistance. Thanks are also due to Dr. T. Kada of National Institute of Genetics for valuable discussion and suggestions.

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