p-Xylylenedicyanide and Related Compounds, Weakly Cytotoxic Nitriles[1]

During investigations[2,3] on the antispasmodic components of *Viburnum* species a fraction from *V. opulus* extract was found to have cytotoxicity. This mixture was the residue after cold extraction of acids and bases from the methylene chloride[2] soluble fraction of the bark. Column chromatography of the mixture using silicic acid and ethyl ether-petroleum ether mixtures gave a crystalline component, m.p. 93–94 °C. A molecular weight of 156 and an elemental formula of $C_{10}H_8N_2$ was obtained from high resolution mass spectrometric data[4], representing a highly unsaturated molecule with 8 rings and/or double bonds. The Table gives the relative abundance data from the low resolution spectrum. These data and the elemental compositions found were consistent with an aromatic dinitrile structure. The nitrile groups were indicated by the ions $(M-HCN)^+$ and $(M-H_2C_2N_2)^+$, while an aromatic ring was suggested by the $C_6H_3^+$, $C_6H_4^+$, and $C_6H_5^+$ fragment ions. The most abundant ion indicated loss of a CH_2CN group, suggesting a cyanohydrocinnamonitrile structure. The mass spectrum of such a structure would be expected to show abundant $(M-CH_2CN)^+$ by benzyl cleavage.

The IR-spectrum (all as potassium chloride pellets) of the compound showed characteristic frequencies at 3020, 1510 and 778 cm^{-1} indicating it to be aromatic, possibly with 1,4-disubstitution. Strong bands were found at 2910, 2925, 1410, 1417 and 745 cm^{-1} indicating methylene functions; an intense nitrile band occurred at 2230 cm^{-1}. The proton magnetic resonance (PMR) spectrum (all in deuterochloroform) was unique in that it contained only 2 peaks, each integrating to 4 protons. The signals fell at 444 and 266 c/sec (tetramethylsilane reference). The UV-spectrum (all in methanol) showed maxima at 266 ($\varepsilon = 109$), 260.5 ($\varepsilon = 139$), 254 ($\varepsilon = 115$) and inflections at 270 and 250 nm.

A synthesis of the spectral data and its elemental composition suggested the compound to be a ring substituted isomer of cyanohydrocinnamonitrile. Consequently, *m*- and *p*-cyanohydrocinnamonitriles were synthesized for comparison and bioassay. The known *o*-cyanohydrocinnamonitrile[5] was eliminated on the basis of physical properties.

To obtain *p*-cyanohydrocinnamonitrile a piperidine catalyzed aldol condensation was run on equimolar quantities of *p*-cyanobenzaldehyde and ethyl cyanoacetate in ethanol. After 45 min the product was filtered and washed to yield 65% of ethyl-α,*p*-dicyanocinnamate, m.p. 171–172 °C (Anal. Calcd. for $C_{13}H_{10}N_2O_2$: C, 69.00; H, 4.42; N, 12.40. Found: C, 69.14; H, 4.64; N, 12.68). The compound was reduced in 3 h using platinum oxide on carbon catalyst and ethyl acetate solvent. The dihydro ester was hydrolyzed without isolation using excess aqueous sodium hydroxide. After acidification, α,*p*-dicyanohydrocinnamic acid was obtained by ether extraction. Crystallization from ethyl acetate-petroleum ether gave the acid in 64% yield, m.p. 128–129 °C (Anal. Calcd. for $C_{11}H_8N_2O_2$: C, 65.99; H, 4.03; N, 13.99. Found: C, 65.89; H, 4.60; N, 14.10). Vacuum decarboxylation of the acid at 175 °C using copper-bronze powder catalyst gave *p*-cyanohydrocinnamonitrile. The dinitrile, m.p. 85–87 °C, was crystallized from carbon tetrachloride in 32% yield (Anal. Calcd. for $C_{10}H_8N_2$: C, 76.95; H, 5.12. Found: C, 77.02; H, 5.46).

The mass spectrum of *p*-cyanohydrocinnamonitrile (Table) was very similar, but not identical, to the spectrum of the unknown. Its IR-spectrum showed frequencies at 3050, 1610, 1510, 1455, 840 and 825 cm^{-1}. Nitrile bands were at 2250 and 2235 cm^{-1}. These were assigned to the aliphatic and aromatic cyano groups on the basis of relative intensities. Methylene group bands were at 2950, 2965, 1425 and 760 cm^{-1}. The PMR-spectrum showed 4 aromatic proton peaks at 467, 458, 450 and 442 c/sec and 4 aliphatic proton peaks at 183, 177, 168 and 162 c/sec. The UV-spectrum showed absorption maxima at 279 ($\varepsilon = 670$), 273 ($\varepsilon = 694$), 270 ($\varepsilon = 680$), 267 ($\varepsilon = 666$), 237 ($\varepsilon = 14{,}800$), and 229 ($\varepsilon = 17{,}500$) nm with shoulders at 265, 262, 259 and 232 nm.

The synthesis of *m*-cyanohydrocinnamonitrile was achieved using an approach analogous to that for the *p*-isomer. Ethyl-α,*m*-dicyanocinnamate, m.p. 123–124 °C, was crystallized in 70% yield from methanol-water (Anal. Calcd. for $C_{13}H_{10}N_2O_2$: C, 69.00; H, 4.42; N, 12.40. Found: C, 68.61; H, 4.49; N, 12.25). The ester was reduced catalytically, hydrolyzed, and decarboxylated to yield 31% of *m*-cyanohydrocinnamonitrile, m.p. 85–88 °C, on crystallization from carbon tetrachloride (Anal. Calcd.

Low resolution mass spectral data for $C_{10}H_8N_2$ isomers[a]. Relative abundance (%)

m/e	*p*-Xylylene dinitrile	*p*-Cyanohydrocinnamonitrile	*m*-Cyanohydrocinnamonitrile
157	2.7	2.1	2.1
156	23.2	18.1	17.8
155	2.6	1.0	1.1
130	1.6	0.2	0.2
129	5.3	1.4	1.3
128	4.1	1.7	1.7
127	1.6	1.1	1.0
117	9.4	9.4	9.4
116	100.0	100.0	100.0
103	2.7	1.5	1.5
102	4.6	2.3	1.9
101	3.3	2.0	1.8
100	1.0	1.1	1.1
90	2.1	4.4	6.6
89	8.8	16.2	15.5
88	2.3	2.3	1.9
87	1.5	1.2	1.1
78	2.0	0.6	0.8
77	5.2	2.6	2.7
76	4.1	4.3	4.2
75	4.1	4.6	4.5
74	2.1	2.0	2.1

[a] Ions below mass 70 have been omitted.

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for $C_{10}H_8N_2$; C, 76.95; H, 5.12; N, 17.95. Found: C, 76.71; H, 5.56; N, 17.73).

The mass spectrum (Table) of *m*-cyanohydrocinnamonitrile differed only slightly from that of the *p*-isomer, but was not identical to that of the unknown compound. Its IR-spectrum showed frequencies at 1598, 1582, 1480, 890 and 800 cm^{-1} as expected. There were 2 nitrile bands, 2245 and 2230 cm^{-1}. As with the *p*-isomer they were assigned to aliphatic and aromatic nitrile groups on the basis of intensities. Methylene group frequencies were found at 2950, 2930, 1430 and 765 cm^{-1}. Contrary to expectation the PMR-spectrum showed a single peak at 455 c/sec equivalent to 4 aromatic protons and 4 aliphatic proton peaks at 182, 177, 167 and 161 c/sec. In view of the aromatic proton spectrum shown by the *p*-isomer a minimum of 4 peaks were expected in the 440 c/sec region. The methyl protons showed as a single peak at 141 c/sec. The UV-spectrum of *m*-cyanohydrocinnamonitrile had maxima at 281.5 ($\varepsilon = 978$), 273 ($\varepsilon = 972$), 225 ($\varepsilon = 12{,}500$) and 232 ($\varepsilon = 11{,}000$) nm and shoulders at 267 and 258 nm.

The close agreement in the mass spectra of the 3 isomeric dinitriles (Table) is similar to, but even more striking than that seen in the mass spectra of ethylbenzene and the xylene isomers[6]. Nor did the model compound IR-spectra assist in eliminating hydrocinnamonitrile structures from consideration. The nitrile region of the references clearly suggested that the unknown was an aromatic nitrile. However, an indication of the unknown substance's structure was found by comparison of its PMR-spectrum with those of the models. The references showed 4 aliphatic proton bands, corresponding to each proton in the side chain but the unknown had a single peak equivalent to 4 protons. These data prompted consideration of the xylylenedicyanides, particularly the *p*-isomer[7]. A comparison of synthetic *p*-xylylenedicyanide with the isolated material showed them to be identical.

When tested for antispasmodic activity using the in vitro rat uterus preparation[2] none of the compounds showed activity. However, when assayed for cytotoxicity, using the Eagle K-B cell tube dilution technique[8], a preliminary screen for antineoplastic effects, the following ID_{50} values obtained: *p*-xylylenedicyanide, 8 μg/ml; *p*-cyanohydrocinnamonitrile, 11 μg/ml; *m*-cyanohydrocinnamonitrile, 25 μg/ml. Although these data indicate a modest degree of cytotoxicity, they introduce the interesting prospect of more potent agents based on the benzylcyanide and hydrocinnamonitrile structures. The unusual structures prompt speculation on possible mechanisms for the cytotoxic effect. In each case the compound could act as an effective nucleophile. If so, the dinitriles are new and relatively non-toxic alkylating agents with possible antineoplastic potential.

Zusammenfassung. Extrakte von *V. opulus* von schwacher Zelltoxizität enthalten *p*-Xylylendinitril. Die Isomeren, *m*- und *p*-Cyanohydrozimtsäurenitrile, wurden synthetisiert und auf ihre zelltoxischen Eigenschaften hin untersucht: *p*-Xylylendinitril erweist sich als am stärksten wirksam.

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A Negative Inotropic Response of Cat Atria to Sympathetic Nerve Stimulation or Norepinephrine

The effects of sympathetic nerve stimulation on cat, rabbit and guinea-pig atria in vitro have been described by many authors[1–8]. None of these reports, however, refer to a negative inotropic response of atria after sympathetic nerve stimulation, an observation we made frequently during recent investigations[9,10] in which innervated cat atria were used. This observation was unexpected in view of the extensive literature describing only positive inotropic and chronotropic effects of sympathetic nerve stimulation or sympathomimetic amines in the mammalian heart. Accordingly, we examined possible causes for this anomalous finding and present here the results of this study.

Isolated atria, with the right cardioaccelerator nerve intact, were prepared by methods described previously[3,9] using cats of either sex. Preparations were maintained under a tension of 1.5–2.0 g in Krebs-bicarbonate solution with the following composition: NaCl, 118.07 m*M*; KCl, 4.75 m*M*; $CaCl_2$, 2.5 m*M*; KH_2PO_4, 0.93 m*M*; $MgSO_4$, 1.19 m*M*; $NaHCO_3$, 25.00 m*M*; glucose, 11.10 m*M*. The fluid was aerated continuously by a mixture of 95% O_2 and 5% CO_2. Rate and force of contraction were monitored by a Grass FT03 force displacement transducer coupled to a Gilson laboratory model polygraph. Changes in force of contraction were considered significant only if they differed from control levels by at least 5%. The nerve was positioned within a double platinum plate shielded electrode located at the surface of the bathing fluid, and

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