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INHIBITION OF MITOCHONDRIAL ELECTRON-TRANSPORT SYSTEMS BY NOR-ISOGUAIACIN

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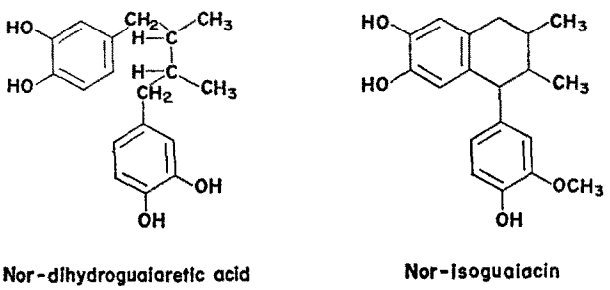
Abstract—The effects of a new constituent isolated from the Creosote Bush, nor-isoguaiacin, on the activities of mitochondrial NADH-oxidase and succinoxidase systems were determined. Nor-isoguaiacin was found to be a potent inhibitor of both mitochondrial electron-transport systems, but did not inhibit cytochrome-oxidase. Preliminary data suggest that nor-isoguaiacin also inhibits energy transfer in rat liver mitochondria.

PREVIOUS reports demonstrated that nor-dihydroguaiaretic acid (NDGA), a component isolated from *Larrea divaricata* Cav. Fam. Zygophyllaceae (Creosote Bush),[1] inhibited heavy beef heart mitochondrial electron-transport enzyme systems[2] and rat liver mitochondrial energy-transfer reactions associated with Site 1 phosphorylation.[3]



Nor-dihydroguaiaretic acid Nor-isoguaiacin

FIG. 1. Structures of nor-dihydroguaiaretic acid and nor-isoguaiacin.

Recently, nor-isoguaiacin, a lignan which is structurally similar to NDGA (Fig. 1), was also isolated from the Creosote Bush.† The pharmacological properties of nor-isoguaiacin have not been reported, but because of its resemblance to NDGA, it represents a pontential inhibitor of the same mitochondrial enzyme systems. The present paper describes some preliminary findings regarding the effect of nor-isoguaiacin on heavy beef heart mitochondrial electron-transport systems and a rat liver mitochondrial energy-transfer system.

METHODS

Heavy beef heart mitochondria (HBHM) were prepared and the activities of the HBHM succinoxidase and NADH-oxidase systems were determined manometrically

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† O. GISVOLD, personal communications.

with and without added nor-isoguaiacin, as previously described.[4,5] Nor-isoguaiacin was added in ethanol which was kept constant in all of the assay flasks (0·1 ml ethanol/3 ml of reaction mixture). In the preliminary studies on energy transfer, rat liver mitochondria were prepared by standard methods,[6] and ADP-stimulated respiration was measured polarographically.[7]

The mitochondrial protein was assayed by the biuret method.[8] Nor-isoguaiacin was kindly supplied by Dr. Ole Gisvold of The University of Minnesota, Department of Pharmacy, who is responsible for its isolation and structural elucidation. *N,N,N',N'*-tetramethyl-*p*-phenlyenediamine (TMPD) was purchased from Eastman Organic Chemicals. Cytochrome *c* type III, β-diphosphopyridine nucleotide, reduced form (NADH), and adenosine diphosphate (ADP) were purchased from The Sigma Chemical Company.

RESULTS AND DISCUSSION

The data presented in Fig. 2 indicate that at a concentration of 75 nmoles/mg of mitochondrial protein, the HBHM succinoxidase enzyme system was totally inhibited.

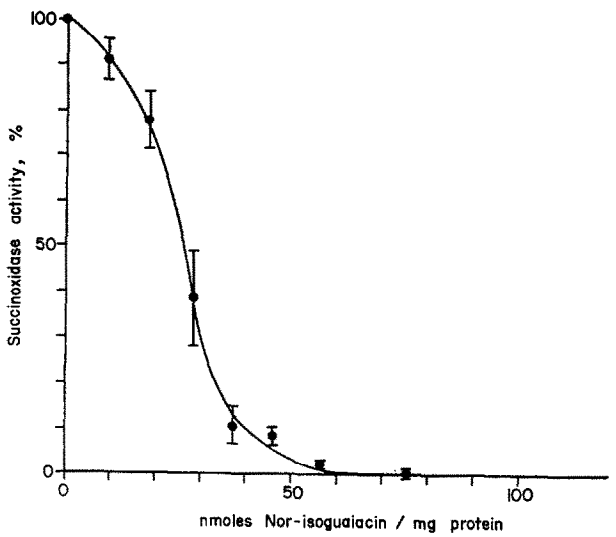

FIG. 2. Titration curve for the inhibition of mitochondrial succinoxidase activity by nor-isoguaiacin. The various points are shown ± the standard error of the mean.

The dose of nor-isoguaiacin required to depress enzyme activity to 50 per cent of the uninhibited controls (I_{50}) was 27 nmoles/mg of protein (Fig. 2). A similar I_{50} value for succinoxidase activity was obtained with nor-dihydroguaiaretic acid (NDGA);[2] consequently, the relative potencies of NDGA and nor-isoguaiacin toward the HBHM succinoxidase enzyme system are comparable. However, nor-isoguaiacin was more efficacious, since at 75 nmoles/mg of protein it totally inhibited succinoxidase activity, whereas at this same concentration NDGA depressed the succinoxidase enzyme system to 10 per cent of the uninhibited controls.[2] Another difference between NDGA and nor-isoguaiacin is that the titration curve for inhibition of HBHM succinoxidase activity is hyperbolic for NDGA and sigmoidal for nor-isoguaiacin. These findings

suggest that nor-isoguaiacin may interact at more than one location in the electron-transport chain.

The data presented in Table 1 demonstrate that nor-isoguaiacin depressed the HBHM NADH–oxidase enzyme system to below 20 and 10 per cent of the uninhibited controls at concentrations of 62·5 and 125 nmoles/mg of mitochondrial protein respectively.

TABLE 1. EFFECT OF NOR-ISOGUAIACIN ON THE BEEF HEART MITOCHONDRIAL NADH-OXIDASE ENZYME SYSTEMS

Additions	Enzyme specific activity (μatoms oxygen consumed/min/mg protein)* I	II	III	Per cent†
None	0·387	0·351	0·272	100
Nor-isoguaiacin				
62·5 nmoles/mg protein	0.064	0.052	0.056	15–20
125 nmoles/mg protein	0·015	0·011	0·022	0–10

* Each flask contained 0·8 mg of mitochondrial protein. Each value represents the average of duplicate samples.

† Per cent of uninhibited controls.

The effect of nor-isoguaiacin on cytochrome-oxidase (ferrocytochrome *c*: oxygen–oxido reductase; EC 1·9·3·1) was assessed by measuring the ability of *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) to bypass the site of inhibition of the HBHM NADH-oxidase system. This approach is based on the principle that exogenous NADH may be nonenzymatically oxidized by TMPD, which in turn shunts electrons back into the electron-transport chain after cytochrome *b*, thereby permitting cytochrome *c* to participate in terminal electron transport via the cytochrome–oxidase pathway; thus in the presence of NADH, TMPD in effect bypasses complexes I and III but not IV. Previous data[2] support this line of reasoning, since the inhibition of the NADH-oxidase enzyme system by rotenone and antimycin, but not by cyanide, was bypassed by TMPD. The data presented in Table 2 demonstrate that the inhibi-

TABLE 2. EFFECT OF *N,N,N′,N′*-TETRAMETHYL-*p*-PHENYLENEDIAMINE (TMPD) ON THE INHIBITION OF MITOCHONDRIAL ELECTRON TRANSPORT BY NOR-ISOGUAIACIN

Additions	NADH-oxidase specific activity (μatoms oxygen consumed/min/mg protein)* I 0	I + TMPD‡	II 0	II +TMPD	Per cent† 0	Per cent† + TMPD
None	0·216		0·272		100	
Nor-isoguaiacin	0·020	0·216	0·061	0·249	8–12	92–100

* Each flask contained 0·8 mg protein. Each value represents the average of duplicate samples.

† Per cent of uninhibited controls.

‡ 125 nmoles/mg protein.

tion of the HBHM NADH-oxidase system caused by nor-isoguaiacin was bypassed by the addition of TMPD. These data are interpreted as indicating that nor-isoguaiacin inhibits mitochondrial electron-transport systems on the substrate side of cytochrome *c*.

The effect of nor-isoguaiacin on energy transfer was assessed on a preliminary basis and the data are presented in Fig. 3. These data demonstrate that NADH-linked, ADP-stimulated respiration was inhibited by the addition of nor-isoguaiacin. This respiratory inhibition was partially released by the addition of dinitrophenol, a finding consistent with the suggestion that nor-isoguaiacin inhibits both electron- and energy-transfer reactions. This finding is not surprising, since NDGA also was reported to inhibit an energy-transfer process[3] in rat liver mitochondria.

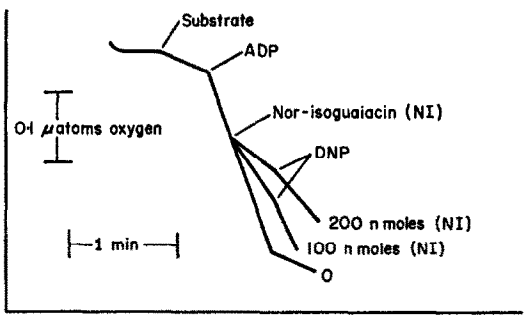

FIG. 3. Effect of nor-isoguaiacin on ADP-stimulated respiration of rat liver mitochondria. The superimposed curves represent different experiments where 0, 100 and 200 nmoles nor-isoguaiacin were added in ethanol (0·05 and 0·1 ml ethanol solution were added for the 100- and 200-nmole addition of nor-isoguaiacin). The buffer (2 ml final volume) consisted of 0·225 M sucrose, 10 mM potassium phosphate buffer, pH 7·4, 5 mM $MgCl_2$, 20 mM KCl and 20 mM Tris buffer, pH 7·4. The mitochondria were added (2·5 mg mitochondrial protein) prior to the addition of substrate which was 5 mM glutamate, 1 mM malate and 1 mM malonate. Respiration was stimulated by the addition of 600 nmoles ADP, and dinitrophenol (DNP) was employed as an uncoupling agent (120 μM). The ADP/O ratio observed with this system was 2·6.

The implication of a Creosote Bush extract and consequently NDGA in the regression of a case of malignant melanoma[9] is consistent with the report[10] that NDGA inhibited aerobic and anaerobic glycolysis and respiration in Ehrlich ascites, K-2 ascites and leukemia L-1210 cells *in vitro*. These authors[10] concluded that NDGA maintained the cellular pyridine nucleotides in such a reduced state that glycolysis was inhibited; however, they did not report on the inhibition of a specific enzyme by NDGA. Pardini *et al.*[2] reported that NDGA inhibited mitochondrial electron-transport systems and suggested that this might alter the cellular pyridine nucleotide redox state in sufficient amounts to inhibit glycolysis. The more recent finding[3] that NDGA also inhibits energy transfer in mitochondria may be of significance for its observed anti-neoplastic effects.[9,10]

Previous studies demonstrated that the lignans, α-peltatin, β-peltatin and podophyllotoxin, exhibited tumor-damaging activity in mice bearing sarcoma 37,[11] and in

acute stem-cell leukemia, lymphosarcoma, mammary adenocarcinoma and melanoma.[12] The extent of tumor damage caused by these lignans was proportional to the extent of inhibition of cytochrome–oxidase activity in sarcoma 37.[13] In addition, a lignan derivative, acetyl podophyllotoxin-*w*-pyridine chloride, inhibitied aerobic metabolism associated with the oxidation of malic, isocitric and succinic acid in sarcoma 37 homogenates.[14,15]

These findings relate the inhibition of mitochondrial electron transport systems by lignans to their anti-neoplastic action. Based on these data, it is tempting to speculate that the lignan, nor-isoguaiacin, could possess anti-neoplastic properties, based on its observed ability to inhibit mitochondrial electron-transport systems and energy transfer. Thus, two Creosote Bush components represents potential cancer chemotherapeutic agents. The effect of nor-isoguaiacin and NDGA on tumor cell metabolism is currently being investigated by our laboratory.

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