TABLE III

POTENTIATING EFFECT OF 2,4-DIAMINOPYRIMIDINES IN COMBINATION WITH SULFISOXAZOLE (SI) AGAINST BACTERIAL INFECTIONS IN MICE

		CD_{50}, mg/kg per os				
Organism	Strain	SI + **3a**[a] potentiation (-fold)	SI + **3b**[a] potentiation (-fold)	SI + **3c**[a] potentiation (-fold)	SI + **3d**[a] potentiation (-fold)	SI + **3e**[a] potentiation (-fold)
Streptococcus pyogenes	4	2.1	3.8	2.6	6.2	1.7
Diplococcus pneumoniae	6301		2.1	2.1		3.8
Staphylococcus aureus	Smith	>5.0	4.0[b]	>3.5	2.5	>11.0
E. coli	257	2.0	5.7[b]	4.7	2.5	>8.9
Klebsiella pneumoniae	KA		3.1	1.7		4.1
Proteus vulgaris	190	2.3	2.6[b]	11.1		9.2
Pseudomonas aeruginosa	B	1.1	1.4	>1.2	0.6	0.8
Salmonella typhosa	P58a		11.0			4.0
Salmonella schottmuelleri			5.4	1.9		1.4

[a] Pyrimidine dose, 50 mg/kg, except [b] 10 mg/kg.

After evapn of Et_2O, the residue was fractioned under vacuum. 3,4,5-Trichlorobenzyl alcohol distilled at 155-170° (11 mm) (10 g, 25%), solidified in the receiver, and melted at 111-112°. 3,4,5-Trichloro-2′-cyanodihydrocinnamaldehyde dimethyl acetal followed at 195-208° (11 mm) (20 g, 35%) and crystd upon standing, mp 85-86°.

2,4-Diamino-5-(3,4,5-trichlorobenzyl)pyrimidine (3d).—3,4,5-Trichloro-2′-cyanodihydrocinnamaldehyde dimethyl acetal (15 g, 0.04 mole) was refluxed with methanolic guanidine (100 ml, 1 *M*) for 2 hr and subsequently the solvent was distilled from an oil bath at 140°. The remaining solid was slurried with H_2O filtered by suction and purified *via* the acetate. The base melted at 285-286°. The compd formed a monohydrate, which was dehydrated upon drying at 100°.

Biological Results.[11]—The *in vivo* antibacterial activities of **3a-e** were tested in mice infected with 100-1000 MLD's of representative Gram-positive and Gram-negative bacteria and treated by oral administration of the respective substances. Compd **3b** protected 50% of the animals infected with *Staphylococcus aureus* Smith, *Escherichia coli* 257, *Klebsiella pneumoniae* KA, *Proteus vulgaris* 190, and *Salmonella typhosa* P58a at doses of 140, 841, 698, 19, and 268 mg/kg, respectively, but was inactive at doses of 1000 to 2000 mg/kg against *Streptococcus pyogenes* 4, *Diplococcus pneumoniae* 6301, *Pseudomonas aeruginosa* B, and *Salmonella schottmuelleri*. Compound **3a** protected 50% of the animals infected with *S. typhosa* P58a at a dose of 177 mg/kg but was inactive at 500-1000 mg/kg against the other organisms tested. No protective effect was detected when **3c-e** were tested at doses of 250-500, 50, and 100 mg/kg, respectively, against any of the 9 bacterial infections.

When the compds were tested *in vivo* at a fixed concn orally of 50 mg/kg (except that **3b** was administered at 10 mg/kg against *S. aureus* Smith, *E. coli* 257, and *P. vulgaris* 190) in combination with graded doses of sulfisoxazole against the bacterial infections, various degrees of potentiation of sulfisoxazole were observed. There was a two-fold or greater increase in the activity of sulfisoxazole against *S. pyogenes* 4 in combination with **3a-d** (2.1-, 3.8-, 2.6-, and 6.2-fold, respectively); against *D. pneumoniae* 6301 in combination with **3b,c,e** (2.1-, 2.1-, and 3.8-fold, respectively); against *S. aureus* 209 in combination with **3a-e** (>5.0-, 4.0-, >3.5-, 2.5-, and >11.0-fold, respectively); against *E. coli* 257 in combination with **3a-e** (2.0-, 5.7-, 4.7-, 2.5-, and >8.9-fold, respectively); against *K. pneumoniae* KA in combination with **3b** and **3e** (3.1-, and 4.1-fold, respectively); against *P. vulgaris* 190 in combination with **3a,b,c,e** (2.3-, 2.6-, 11.1-, and 9.2-fold, respectively); in combination with **3b** and **3e** against *S. typhosa* P58a (11.0- and 4.0-fold, respectively) and in combination with **3b** against *S. schottmuelleri* (5.4-fold). No potentiation of sulfisoxazole was observed with any compound against *P. aeruginosa*. These results are summarized in Table III.

Acknowledgment.—The microanalyses were obtained by Dr. F. Scheidl and his associates of our Microanalytical Laboratory. The nmr spectra were obtained by Dr. T. Williams of our Physical Chemistry Department. We gratefully acknowledge the technical assistance of Mr. Sam Gruenman.

(11) The *in vivo* test methodologies may be found in E. Grunberg and W. F. DeLorenzo, *Antimicrob. Ag. Chemother.*, **1966**, 430 (1967).

Seeds as Sources of L-Dopa[1]

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The L isomer of dopa [3-(3,4-dihydroxyphenyl)alanine] is being used for symptomatic relief of Parkinson's disease.[2] It is presently obtained by synthesis or by processing fish flour.[3] A patent has been issued[4] for its preparation from velvet bean seed. Since the isolation of dopa from *Vicia faba* in 1913,[5] the compound has been reported in plant parts of species of the legumes *Baptisia, Lupinus, Mucuna* (including *Stizolobium*), and *Vicia* at levels up to 1.9%.[4,6-8] The compound has also been reported in the *Euphorbiaceae* as 1.7% of the fresh weight of the latex of *Euphorbia lathyrus*[9] and in the latex from *Euphorbia dendroides*.[10]

In the course of a survey in which amino acids in seed meals were determined by ion-exchange chromatography of acid hydrolysates, an unidentified peak eluting after leucine[11,12] was observed. The elution position of

(1) Presented at Division of Medicinal Chemistry, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(2) J. E. Randal, *Today's Health*, **48**, 34 (1970).

(3) *Chem. Eng. News*, **48**, 43 (Jan 26, 1970).

(4) Don V. Wysong, Dow Chemical Co., U. S. Patent 3,253,023 (1966); *Chem. Abstr.*, **65**, 5529a (1966).

(5) M. Guggenheim, *Z. Physiol. Chem.*, **88**, 276 (1913); *Chem. Abstr.*, **8**, 1128 (1913).

(6) G. Just, J. Kagan, and T. J. Mabry, personal communication, 1970.

(7) M. Damadoran and R. Rasaswamy, *Biochem. J.*, **31**, 2149 (1937).

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(9) I. Liss, *Flora (Jena)*, **151**, 351 (1961); *Chem. Abstr.*, **57**, 3786e (1961).

(10) M. Adinolfi, *Rend. Accad. Sci. Fis. Mat., Naples*, **31**, 335 (1964); *Chem. Abstr.*, **64**, 3961g (1964).

(11) C. H. VanEtten, R. W. Miller, I. A. Wolff, and Q. Jones, *J. Agr. Food Chem.*, **11**, 399 (1963).

(12) C. H. VanEtten, W. F. Kwolek, J. E. Peters, and A. S. Barclay, *ibid.*, **15**, 1077 (1967).

this peak was reported[13] and later confirmed in our laboratory to be identical with that of authentic L-dopa. Based on the appearance of this peak, seed from four species of legumes, *Baptisia leucantha, Dolichos lablab, Mucuna deeringiana,* and *Vicia faba,* were the only ones of 379 species analyzed that contained dopa. By calculations from the peak area, the seed from *M. deeringiana* contained 3.0% and the remaining three species less than 0.5%. Current interest in the compound prompted us to search for other, possibly richer, sources.

Tlc was used as the primary tool in screening the seed extracts for detection of dopa. In the manner described, dopa could be detected in the extracts of seeds containing amounts of the compound in excess of about 0.5%. A uv absorption procedure was used for quantitative measurements. It proved to be a rapid and reliable means of estimating the compound in seed of the *Mucuna* species.

A total of 724 species from 447 genera of 135 families have been screened by the tlc procedure. About 50% of the genera and 75% of the species were in 7 of the 135 families. These 7 families and the number of genera within them that were examined are as follows: *Leguminosae*, 145; *Boraginaceae*, 29; *Euphorbiaceae*, 26; *Compositae*, 22; *Labiatae*, 9; *Gramineae*, 8; and *Lauraceae*, 7. Nearly all the remaining families were represented by 1–3 species from each. One large family, the *Cruciferae,* was not tested because a large number of crucifer species were previously analyzed for amino acids[14] and dopa was not found on reexamination of the data.

The legume family received the greatest attention in our survey with 332 species because (1) the first isolation of L-dopa was from *V. faba*,[5] and (2) 1–3% amounts of the compound were found in seed of some species of *Mucuna*.[4,7,8,12]

None of the seed from 30 species of *Vicia* examined contained enough dopa to give a positive test by the method used. One of 3 accessions of *V. faba* was also analyzed by the more sensitive ion-exchange amino acid analyzer and contained less than 0.1% dopa.

Sesame seed (*Sesamum indicum*) reportedly contains some dopa.[2] Seed from sesame, from *Rogeria longiflora* (both from the family *Pedaliaceae*), and from 2 species of the closely related family *Martyniaceae,* which is sometimes included as a part of the *Pedaliaceae,* did not contain enough dopa to be detected. Examination of seed from 52 species of *Euphorbia* gave negative results, although the compound is reported in the latex from 2 species.[9,10]

The currently examined 724 species, when combined with the previous species examined by amino acid analyses,[11,12,14] represent a total of 1062 different species from 160 plant families.

Dopa isolated in 2.3% yield from *M. deeringiana* gave an ir spectrum in good agreement with that from the reference material, and its rotation, $[\alpha]_D$ $-8.37°$ (c 2.0, 1 N HCl), compared favorably with the rotation, $[\alpha]_D$ $-8.81°$, reported in the patent.[4]

Only in seed of the genus *Mucuna* were amounts found above 0.5% (Table I). The large amounts of the compound found in the *Mucuna* samples and the failure to find corresponding amounts in other species suggested that members of this genus are the best seed sources.

TABLE I

DOPA CONTENT OF MUCUNSA SPECIES

Species	Dopa, %[a]	Origin
M. aterrima	5.0	Mexico
M. aterrima	4.3	Florida
M. aff. *aterrima*	4.4	Colombia
M. aff. *aterrima*	4.6	Costa Rica
M. aff. *aterrima*	4.7	Nigeria
M. aff. *deeringiana*	4.8	Louisiana
M. aff. *deeringiana*	4.9	Rhodesia
M. holtonii	6.7	Guatemala
M. urens	5.2	Florida
M. sp.	3.1	Georgia
M. sp.	4.4	Japan

[a] In defatted, air-dried seed meal.

The genus *Mucuna* contains an estimated[15] 160 species of scandent herbs or shrubs distributed in both the New and Old World tropics and subtropics. Taxonomically, the species of *Mucuna* have been divided into 3 or 4 sections or subgenera. The only economically important species of *Mucuna* are the velvet beans belonging to the section *Stizolobium,* a group considered by some taxonomists to constitute a separate genus. Velvet beans are viny or bush-like annual herbs that are cultivated in various warm regions of the world for fodder, green manure, and for their edible seeds.

With the exception of *M. holtonii* and *M. urens*, both of which are wild tropical lianas, the *Mucuna* species included in the present survey are cultivated velvet beans. Although percentages of dopa are highest in *M. holtonii* and *M. urens,* these species, because of their wild habit, have little to recommend them as potential seed sources of the compound. The variability in dopa content exhibited in our limited sampling of the cultivated velvet beans suggests that high-yielding strains might be developed through breeding and selection.

Experimental Section

A 500-mg sample of finely ground, defatted seed meal was heated on a steam bath with 5 ml of 0.1 N HCl for 5 min. After cooling, the mixt was shaken vigorously with 10 ml of EtOH and centrifuged. For tlc, 1 ml of the ext was concd to 0.5 ml, and a 5- to 10-μl portion was spotted on a 20 × 20 cm plate of silica gel G[16] (0.25-mm layer). The air-dried plate was developed with i-PrOH–EtOAc–H_2O–AcOH (20:19:10:1), and the solvent front was allowed to migrate 12.5 cm above the point of sample application. When the plate was sprayed with ninhydrin reagent (0.5% in n-BuOH–Me_2CO 1:1) and heated 10 min at 110°, dopa (R_f 0.55) gave a characteristic gray-blue spot. Two spots of known dopa were applied to each plate as reference material for comparison of color and migration. If dopa was detected in an extract by tlc, the amt present was estimated by measuring the uv absorption at 283 nm. An ext, prepd as described above, was decanted into a 200-ml volumetric flask and an additional 25 ml of EtOH added to the residue meal. After vigorous shaking, the sample was again centrifuged and the alcohol wash was decanted into the volumetric flask with the initial ext and made to vol with EtOH. The added EtOH usually caused further pptn, so a portion of the soln was filtered for measurement of uv absorption.

A calibration curve prepd with known dopa was a straight line up to 100 μg of dopa/ml, the highest concn measured. Since

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(15) J. Hutchinson, "The Genera of Flowering Plants, Dicotyledons 1," The Clarendon Press, Oxford, 1964, pp 433–434.

(16) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

exts from seed meals may be expected to exhibit nonspecific absorption in the uv, it was considered likely that a correction would be necessary. Minima on either side of the λ_{max} 283 peak occurred near 255 and 305 nm. To provide the desired correction, absorbances at 255 and 305 nm were averaged and subtracted from the absorbance at the maximum to give a net value. From our measurements a net absorbance of 1.000 was equiv to 65 μg/ml. Confidence in the uv absorption as a rapid means of quantitation was gained by comparison of the value (6.7%) obtained for *M, holtonii* with that calcd for *M. holtonii* when detd on the ion-exchange analyzer (6.2%).

Dopa was isolated from *M. deeringiana* by the patented process.[4] The dopa used for reference and calibration measurements was from Mann Research Laboratories. Uv measurements were made with a Beckman Model DK-2a recording spectrophotometer.

The names of the 135 families, 447 genera, and 724 species examined in the present work are available from the authors on request.

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New Compounds

2-(5-Nitro-2-thienyl)cinchoninic Acids

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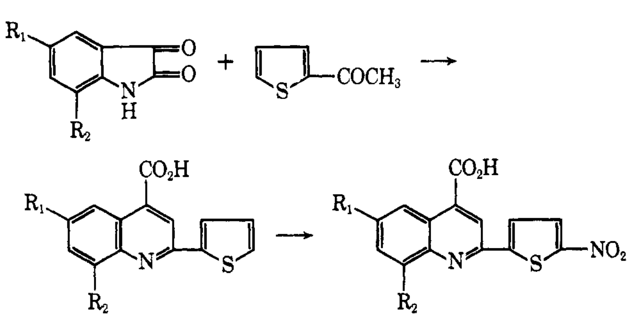
The antibacterial activities of 2-(5-nitro-2-furyl)cinchoninic acid and derivatives have been reported.[1] In a search for more potent antibacterial compounds, we have been preparing a series of their S analogs, 2-(5-nitro-2-thienyl)cinchoninic acids.



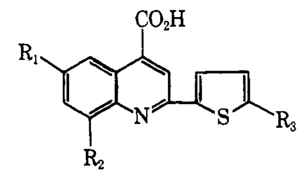
Preliminary *in vitro* tests of the compounds prepared, against *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhosa*, and *Staphylococcus album* did not show significant activity.

Experimental Section[2]

2-(2-Thienyl)cinchoninic Acids.—A mixture of 0.02-mole quantities of an appropriate isatin and 2-acetylthiophene in 15 ml of aq 20% KOH and 15 ml of EtOH was heated under reflux for 12 hr. The reaction mixt was cooled and acidified with dil HCl and the resulting yellow ppt was removed by filtration and crystd from AcOH (See Table I).

2-(5-Nitro-2-thienyl)cinchoninic Acids.—To a cold soln of 0.01 mole of 2-(2-thienyl)cinchoninic acid in 15 ml of concd H_2SO_4, 3 ml of a mixt of concd H_2SO_4 and concd HNO_3 (1:1) was added with vigorous stirring. After 1 hr, 200 g of crushed ice was added to the reaction mixt and the resulting ppt was filtered and crystd from AcOH. The positions of the NO_2 groups were confirmed by nmr spectroscopy (DMSO). (See Table I.)

TABLE I



No.	R_1	R_2	R_3	Yield, %	Mp, °C dec	Formula[a]
1	H	H	H	80	210[b]	$C_{14}H_9NO_2S$
2	H	H	NO_2	63	280	$C_{14}H_8N_2O_4S$
3	F	H	H	79	250	$C_{14}H_8FNO_2S$
4	F	H	NO_2	84	299	$C_{14}H_7FN_2O_4S$
5	Cl	H	H	73	261	$C_{14}H_8ClNO_2S$
6	Cl	H	NO_2	85	293	$C_{14}H_7ClN_2O_4S$
7	Br	H	H	95	250	$C_{14}H_8BrNO_2S$
8	Br	H	NO_2	74	262	$C_{14}H_7BrN_2O_4S$
9	CH_3	H	H	90	222	$C_{15}H_{11}NO_2S$
10	CH_3	H	NO_2	78	308	$C_{15}H_{10}N_2O_4S$
11	H	CH_3	H	82	242	$C_{15}H_{11}NO_2S$
12	H	CH_3	NO_2	91	282	$C_{15}H_{10}N_2O_4S$

[a] All compds were analyzed for C, H, and the anal. results were satisfactory. All compds were subjected to nmr and ir spectroscopy. The spectroscopic data were as expected. [b] Lit. [P. Schaefer, K. S. Kulkarni, R. Costin, J. Higgins, and L. M. Honig, *J. Heterocycl. Chem.*, **7**, 607 (1970)] gives mp 209–211°.

Acknowledgments—The authors gratefully acknowledge the constant encouragement of Professor A. Zargari of Tehran University.

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(2) Melting points were taken on a Kofler hot stage microscope and were uncorrected. The ir spectra were determined with a Leitz Model III spectrograph. Nmr spectra were obtained on a Varian A60A instrument.

Analogs of Albizziin

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The experimental and clinical use of asparaginase as as antitumor agent[1] has led to a renewed interest in the synthesis of analogs of asparagine. Albizziin, L-2-amino-3-ureidopropionic acid,[2] contains an NH group

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