Phytochemistry, 1978, Vol 17, pp 1083–1087 © Pergamon Press Ltd Printed in England 0031-9422/78/0701-1083$02.00/0

THE RELATIONSHIP BETWEEN L-DOPA DECARBOXYLASE IN THE LATEX OF *PAPAVER SOMNIFERUM* AND ALKALOID FORMATION*

MARGARET F. ROBERTS and MIKHAIL D. ANTOUN†
Department of Pharmacognosy, The School of Pharmacy, London University, 29/39 Brunswick Square, London WC1N 1AX, U.K.

(*Received* 19 *December* 1977)

Key Word Index—*Papaver somniferum*; Papaveraceae; L-dopa decarboxylase; alkaloid biogenesis; secondary metabolism.

Abstract—The presence of L-dopa decarboxylase has been demonstrated in poppy latex utilising L-dopa-1-[^{14}C] and L-dopa-3-[^{14}C] as substrates. The enzyme appeared to have maximum activity at pH 7.2 and showed both substrate and pyridoxal phosphate inhibition. The substrates L-tyrosine, L-phenylalanine and L-histidine were also decarboxylated. L-dopa decarboxylase was found to occur solely in the latex supernatant fraction. The possible involvement of this enzyme in alkaloid biosynthesis in the latex is discussed.

INTRODUCTION

The involvement of L-tyrosine, L-dopa and dopamine in the biosynthesis of the opium alkaloids is well documented [1]. The recent experiments of Wilson and Coscia [2] in which dopamine- [1-^{14}C-2-^{3}H], dopa-[2-^{14}C], dopa-[1-2-^{14}C], dopa-[carboxy-^{14}C] and norlaudanosoline carboxylic acid-[3-^{14}C-4-^{3}H] were introduced into seedlings and latex of *P. orientale* suggests the formation of norlaudanosoline from dopamine and 3,4-dihydroxyphenylpyruvic acid via the intermediates norlaudanosoline carboxylic acid and 1,2-dehydronorlaudanosoline. The results infer that L-dopa may be metabolised in *P. orientale* in at least two ways, decarboxylation to dopamine and transamination to 3,4-dihydroxyphenylpyruvic acid. Further confirmation of these results has been obtained by Battersby *et al.* [3] with *P. somniferum*. However these workers noted that externally introduced dopa-[2-^{14}] did not significantly label the pool of 3,4-dihydroxyphenylpyruvate from which the amino acid norlaudanosoline carboxylic acid is presumably formed.

The two key enzymes involved in the initial phases of alkaloid biosynthesis, a decarboxylase and a transaminase have been shown to occur in acetone powder preparations of *P. somniferum* roots [4] though the report of L-dopa decarboxylase was later challenged [5]. The occurrence of L-dopa decarboxylase (3,4-dihydroxyphenyl-alanine carboxylase EC 4.1.1.26) in plants might be inferred from the numerous reports of dopamine [6–8]. However, the reports of the occurrence of L-dopa decarboxylase (L-dopa D.C.) are few and challengeable [4, 9].

Fairbairn *et al.* [10, 11] has shown that the alkaloids in *P. somniferum* occur within the 1000 *g* fraction (alkaloid vesicles) of the latex and has also suggested that their biogenesis takes place within these organelles. However our recent experiments [12] on a methyltransferase in poppy latex and preliminary investigations of L-dopa D.C. [13] indicate that these activities occur in the supernatant fraction of the latex (latex minus 1000 *g*/30 min fractions). The present work therefore investigates the occurrence and location of L-dopa D.C. in poppy latex in relation to alkaloid formation in the vesicles.

RESULTS

Detection of L-*dopa D.C. in* P. somniferum *latex*

Preliminary experiments with isolated poppy latex utilised L-dopa-1-[^{14}C] and $^{14}CO_2$ evolution was used as a measurement of L-dopa D.C. This method was utilised for convenience in the determination of substrate specificity. However the high background radioactivity due to non-enzymic oxidation of L-dopa made it preferable to use a method which involved the isolation of the reaction product dopamine. For most experiments therefore L-dopa-3-[^{14}C] was used and the dopamine formed separated from excess L-dopa by ion exchange chromatography on Amberlite CG50. Further verification of the production of dopamine was made by PC. Since it has been observed that the carboxyl group of dopachrome, an oxidation product of L-dopa, is labilised in neutral solutions by anions to produce 5,6-dihydroxyindole [14], authentication of dopa in these experiments was essential.

It was found that L-dopa D.C. was only detectable in poppy latex up to approximately one week from petal opening and it is possible that alkaloid biosynthesis in the latex gradually ceases as the capsule develops. The inclusion of the monamine oxidase inhibitors paraglyline and tranycypromine at 0.1 and 0.2 μM respectively had negligible effect on activity. However the inclusion of 20 mM ascorbate and 10 mM DIECA considerably reduced non-enzymic oxidation and oxidation due to phenolase. These substances were therefore routinely included in the assay mix. The decarboxylation of L-dopa as measured by dopamine formation was almost linear over the first 20 min. The pH max for the formation of

* Part 6 of 'Enzymic Studies with *Pavaver somniferum*'; for Part 5 see ref. [11].

† Present address: Dept. of Pharmacognosy, Faculty of Pharmacy, P.O. Box 1996, Khartoum, Sudan.

dopamine appeared to be at 7.2 although problems with the stability of L-dopa in alkaline solutions made accurate measurement of pH above 7.2 difficult.

The occurrence of L-dopa D.C.

Experiments with poppy latex separated into a 1000 *g*/30 min fraction (alkaloid vesicles) and the supernatant revealed the apparent production of dopamine by both fractions though radioactivity was low in the control samples with boiled latex. However, it was found (Table 1) that the activity in the 1000 *g*/30 min was virtually completely inhibited by the inclusion of 10 mM DIECA whereas this had little effect on the activity of the supernatant fraction. Further, PC showed that the radioactivity with the 1000 *g*/30 min fraction did not coincide with the dopamine whereas with supernatant samples the dopamine was radioactive. Previous work [15] has shown that the latex phenolase resides in the 1000 *g*/30 min fraction and it was therefore assumed that the apparent activity of this fraction was the result of the formation of dopachrome by this enzyme complex. The inhibitory effect of DIECA, a copper chelator, on the activity in the 1000 *g*/30 min fraction would further substantiate this view.

Table 1. The occurrence of L-dopa decarboxylase in *P. somniferum* latex

Poppy latex fraction	Protein mg/ml latex	Additives	L-dopa decarboxylase activity (dpm/ml latex/min)
1000 *g*/30 min	0.5	—	11049
		+ DIECA	
1000 *g*/30 min	0.5	+ ascorbate	815
		+ DIECA	
1000 *g*/30 min (boiled)	0.5	+ ascorbate	560
Supernatant	1.0	—	14400
Supernatant		+ DIECA	
	1.0	+ ascorbate	14200
		+ DIECA	
Supernatant	1.0	+ ascorbate	450

The complete system contained poppy latex fraction (0.05 ml) L-dopa-3-[^{14}C] 0.2 ml (1.8 mCi/mmol), DIECA 1 mM, ascorbate 20 mM and 100 mM phosphate 500 mM mannitol buffer pH 7.2 to a total vol. of 0.5 ml. Latex fractions were preincubated 10 min at 25° with the additive. The reaction was initiated with the substrate and incubation was at 35° for 20 min.

In view of the results of Fairbairn *et al.* [10, 11] in which incorporation of L-dopa-3-[^{14}C] into morphine appeared to take place in the alkaloidal vesicles and our previous results indicating that the methylation of norlaudanosoline takes place in the supernatant [12], further fractionation of the latex was considered essential since the two groups of researchers had used slightly different centrifuge programmes for the sedimentation of the alkaloid vesicles. A further fractionation of the supernatant to give 4500 *g*/30 min and 10000 *g*/30 min fractions was made. Table 2 shows clearly that L-dopa occurs only in the supernatant fraction and therefore the suggestion that contamination of the alkaloid vesicle fraction with slightly heavier particles containing L-dopa D.C. did not explain the different results obtained in the two groups.

Table 2. The distribution of L-dopa decarboxylase in *P. somniferum* latex

Poppy latex fraction	Protein mg/ml latex	L-dopa D.C. activity (dpm/ml latex/min)
100 *g*/30 min	0.44	1000
4500 *g*/30 min	0.05	negl.
10000 *g*/30 min	0.05	negl.
Supernatant	0.98	11900

The complete system contained poppy latex fraction (0.05) L-dopa-3-[^{14}C] 0.2 mM (1.8 mCi/mmol), DIECA 1 mM, ascorbate 20 mM and 100 mM phosphate 500 mM mannitol buffer pH 7.2 to a total vol. of 0.5 ml. The latex fractions were preincubated with the additives in buffer for 10 min at 25°. The reaction was initiated with the substrate and incubation was at 35° for 20 min.

The effect of substrate and pyridoxal phosphate concentration on L-dopa D.C.

Preliminary experiments indicated that high concentrations of the substrate L-dopa were inhibitory. It was also shown that pyridoxal phosphate (PLP) strongly inhibited activity at concentrations of 1 mM. A study was therefore made of the effect of substrate and PLP concentration on L-dopa D.C. activity. The results given in Table 3 and Fig. 1. show that in the absence of PLP under the experimental conditions used, L-dopa becomes inhibitory at concentrations above 0.2 mM where maximal activity is observed (Fig. 1.). At this concentration of L-dopa, 1 mM PLP inhibited L-dopa D.C. 66% whereas 0.1 mM PLP gave only 12% inhibition and 0.01 mM PLP showed considerably increases in L-dopa D.C.

Table 3. The effect of pyridoxal phosphate and inhibitors on *P. somniferum* latex L-dopa decarboxylase

Complete system + the following additives (mM)		% activity
Control		100
KCN	1.0	8
	0.1	34
Na semicarbazide	1.0	58
	0.1	92
Hydroxylamine	1.0	6
	0.1	10
Isoniazid	5.0	81
	50.0	18
Na iodoacetate	1.0	88
	0.1	92
p-CMB	1.0	52
	0.1	84
pyridoxal phosphate	1.0	33
	0.1	88
	0.01	170

The complete system contained poppy latex supernatant ≈600 μg protein (0.05 ml) L-dopa-3-[^{14}C] 0.2 mM (1.8 mCi/mmol); DIECA 1 mM, ascorbate 20 mM and 100 mM phosphate 500 mM mannitol buffer pH 7.2 to a total vol. of 0.5 ml. The latex supernatant was preincubated 10 min at 25° in buffer containing the relevant additives. The reaction was initiated with the substrate and incubation was at 35° for 20 min.

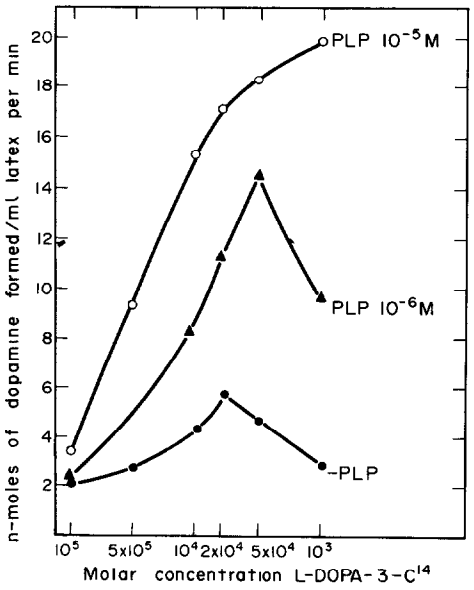

Fig. 1. The effect of the concentration of L-dopa and pyridoxal phosphate on L-dopa D.C. The complete system contained poppy latex supernatent 600 μg protein (0.05 ml) L-dopa-3-[^{14}C], 0.2 mM (1.8 mCi/mmol), DIECA 10 mM, ascorbate 20 mM, and 100 mM phosphate 500 mM mannitol buffer pH 7.2 to a total vol. of 0.5 ml. The latex supernatant was pre-incubated with the additives 10 min at 25°. The reaction was initiated with the L-dopa-3-[^{14}C] and incubation was for 20 min at 35°.

activity (170%). With lower concentrations of L-dopa (0.005 mM) 1 mM PLP completely inhibited L-dopa D.C. In a study of the effect of variation in substrate concentration for two concentrations of PLP stimulation of activity by (0.001 mM) PLP was observed for substrate concentrations up to 0.05 mM thereafter some inhibition was observed. However with 0.01 mM PLP no inhibition of decarboxylase activity was observed with the concentrations of substrate used.

The effect of enzyme inhibitors on L-dopa decarboxylase

The effect of a number of enzyme inhibitors on L-dopa D.C. was studied (Table 3). The inhibition by *p*-chloromercuribenzoate (*p*-CMB) and iodoacetate gives some indication of the involvement of sulphydryl groups. The enzyme was also inhibited by KCN, semicarbazide and hydroxylamine indicating the presence of a carbonyl function presumably pyridoxal phosphate since inhibition with a specific pyridoxal phosphate inhibitor, isoniazid [16] was also observed. The inhibition with isoniazid at 5 and 50 mM was similar to that for L-dopa D.C. in animals [17].

Substrate specificity of poppy latex L-dopa decarboxylase

Using carboxyl-[^{14}C] substrates it was possible to determine the molar amounts of substrate decarboxylated by measurement of the $^{14}CO_2$ evolved. A study of the decarboxylation of L-phenylalanine, L-tyrosine and L-histidine showed that the rate of decarboxylation of these substrates was very low compared with that of L-dopa.

Table 4. Substrate specificity of *P. somniferum* latex L-dopa decarboxylase

Substrate	Substrate decarboxylated latex/min $^{14}CO_2$ nmol/ml
L-dopa-1-[^{14}C] (18 mCi/mmol)	0.7
L-phenylalanine-1-[^{14}C] (59 mCi/mmol)	0.001
L-tyrosine-[U-^{14}C] (5 mCi/mmol)	0.03
L-histidine-1-[^{14}C] (5 mCi/mmol)	0.04

The complete system contained poppy latex supernatant ≈ 1 mg protein (0.1 ml) substrate 0.2 mM, DIECA 1 mM, ascorbate 20 mM and 100 mM phosphate 500 mM mannitol buffer pH 7.2 to a total vol. of 1.0 ml. The latex supernatant was pre-incubated 10 min at 25° with the DIECA, ascorbate and buffer and the reaction initiated with the substrate. Incubation was at 35° for 20 min. L-tyrosine-[U-^{14}C] was assumed to have equally labelled carbon atoms.

DISCUSSION

Records for the occurrence of L-dopa D.C. in higher plants are few [4, 7] and its detection is difficult in part due to the instability of L-dopa but also because most higher plants contain phenolases. The phenolic oxidation of L-dopa to dopachrome both nonenzymically and as a result of phenolase activity is well established [18] and makes essential the inactivation of these enzymes prior to any attempted estimation of L-dopa D.C. It proved fortuitous in experiments with poppy latex that all the phenolase activity was isolatable with the 1000 *g*/30 min fraction (alkaloid vesicles) whereas the L-dopa D.C. proved to be in the supernatant. Further it was found possible to completely inhibit phenolase with 10 mM DIECA while D.C. activity remained unaltered. The results obtained would therefore appear to substantiate the earlier record for L-dopa decarboxylase in *P. somniferum* seedlings [4] but casts some doubt on the isolation of L-dopa D.C. from *Cytisus scoparius* [7] where the enzyme appears to be strongly inhibited by DIECA and where little stimulation of activity is observed with PLP. We would suggest therefore that the $^{14}CO_2$ evolution observed in these particular experiments is due to oxidative decarboxylation of L-dopa resulting from phenolase activity.

Experiments with enzyme inhibitors indicated a requirement for PLP. However preliminary experiments with both L-dopa and PLP showed that both inhibited L-dopa D.C. at high concentrations although low concentrations (0.01–0.001 mM) of PLP stimulated activity 3–4 fold.

L-dopa is known to readily form L-dopa melanin and this substance has been shown to inhibit L-dopa D.C. in rat liver systems [19]. It is therefore possible that substrate inhibition with poppy latex L-dopa D.C. is the result of the formation of L-dopa melanin. It has also been shown [19] that in the presence of PLP, an L-dopa melanin–PLP complex is formed which also inactivates L-dopa D.C. in rat liver. Other researchers [20–22] have shown that a Picket–Spengler condensation between L-dopa and PLP may take place to produce the tetrahydroisoquinoline alkaloid L-dopa–PLP which readily inhibited aromatic L-amino acid decarboxylase, and it has been suggested [22] that this is one of the principle causes for inhibition of L-dopa D.C. by

PLP. These workers also suggest that high concentrations of PLP may be more favourable to L-dopa melanin formation. The results given in Table 3 and Fig. 1. would seem to suggest that in higher plants and poppy latex in particular, L-dopa D.C. is also inhibited by the formation of these condensation products. Inhibition by high concentrations of L-dopa in the absence of PLP could be principally due to L-dopa melanin formation. In the presence of 0.001 and 0.01 mM PLP, L-dopa D.C. inhibition requires somewhat greater concentrations of the substrate. This may result either from PLP protecting the active site or may depend on a reduction in L-dopa melanin formation due to L-dopa–PLP formation, the implication being that the latter is less inhibitory at low concentration than L-dopa melanin. The severe inhibition of L-dopa D.C. with higher concentration of PLP is probably largely due to the Picket–Spengler condensation product L-dopa–PLP. However without further data the effect of the various L-dopa products on L-dopa D.C. and the stimulation by PLP at low concentrations must remain uncertain. The fact that the activity found in the supernatant fraction of poppy latex does show this sensitivity both to substrate and PLP concentration would appear to further substantiate its identity as L-dopa D.C. rather than phenolic oxidative decarboxylation.

Whilst it has been suggested that the enzyme responsible for the decarboxylation of L-dopa is sufficiently broad to warrant the name 'aromatic L-amino acid decarboxylase' [23, 24] some controversy has surrounded the activity of the enzyme towards L-tyrosine and L-histidine [25–27]. Recent work [29] however substantiates the work of Lovenberg *et al.* [24] though activity towards these amino acids with most animal systems is low compared with L-dopa. The experiments with poppy latex give similar results.

Finally it is necessary to consider the role of L-dopa D.C. in alkaloid formation in *P. somniferum*. Recent work [2, 3] gives a clear indication of the involvement of L-dopa D.C. in the formation of the initial condensation product norlaudanosoline carboxylic acid and the results in ref. [2] suggest a ratio for L-dopa D.C. to transaminase of 78/11. Early work discussed in ref. [1] indicates that alkaloid biosynthesis takes place in the latex and work by Fairbairn *et al.* [10, 11] suggests that the biosynthesis of the alkaloids takes place in the alkaloid vesicles where the alkaloids are stored and further metabolised. The work recorded in ref. [10] shows that morphine-[$^{14}C_3$] is actively taken up by the alkaloid vesicles (96% in 1.5 hr) and this has been further substantiated by our separation of these organelles on a sucrose gradient to show that the morphine-[$^{14}CH_3$] is taken up by the organelles which contain the phenolase [28]. However our results on the methyltransferase [11] responsible for the formation of reticuline from norlaudanosoline and the present L-dopa D.C. experiments strongly suggest that alkaloid biosynthesis takes place in the supernatant rather than the 1000 *g*/30 min alkaloid vesicles. Although originally [11] we considered that due to differences in the centrifuge programmes used by the two groups, the enzymes responsible for alkaloid biosynthesis were possibly located in particles somewhat lighter than the 1000 *g*/30 min fractions but not always clearly separated from it. The present results show clearly that this is not the case. We therefore suggest that the alkaloids are formed in the supernatant fraction of the latex and are subsequently taken up by the 1000 *g*/30 min alkaloid vesicles where they accumulate.

EXPERIMENTAL

Preparation of P. somniferum *latex.* Latex was collected as previously described [15] in 500 mM mannitol 100 mM phosphate buffer pH 7.2 such that the latex: buffer ratio was 1·1.

Isolation of the 1000 g *organelles.* Latex was centrifuged at 1000 g for 30 min and the resulting sediment washed once with an equal vol. of buffer and then resuspended in a vol. of buffer equal to the original latex:buffer vol. The rest of the latex was termed the 'supernatant' and unless otherwise stated this term will be used to refer to the whole latex minus the 1000 *g* organelles.

Further fractionation of the latex supernatant. For some experiments the 'supernatant' was further fractionated by centrifugation at 4500 *g*/30 min and 10000 *g*/30 min. The remainder of the latex in these experiments was considered as the supernatant.

Assays for L-*dopa decarboxylase*: (a) *Determination of activity by the isolation of dopamine.* The reaction mixture was prepared in Eppendorf tubes immersed in ice, with the following components, ascorbate 20 mM, DIECA 10 mM, 500 mM mannitol, 100 mM phosphate buffer pH 7.2 to a total vol. of 0.5 ml, latex prepn (0.05 ml) and L-dopa-3-[^{14}C] 0.2 mM (1.8 mCi/mmol) or 0.05 mM (9.2 mCi/mmol). The latex prepn was pre-incubated 10 min at 25° with buffer, DIECA and ascorbate and the reaction initiated with L-dopa. Incubation was at 35° for 20 min. The reaction was stopped with 3% sulphosalicylic acid (0.2 ml), 5% EDTA (0.2 ml), 5% Na metabisulphite (0.2 ml) and 200 mg/ml dopamine (0.01 ml). Samples were stored immediately at −20° until the assay could be completed. Dopamine was separated from unreacted L-dopa by ion exchange chromatography. The pptd protein was first removed from the extracts which were then buffered to pH 6.2 with 3N NH_4OH. The buffered soln was made to 3 ml and transferred to a small (0.6 × 4 cm, 0.5 g) amberlite CG 50 column (NH_4^+ form buffered to pH 6.2). The column was washed with 0.2 M ammonium acetate buffer pH 6.2 (15 ml) to remove excess L-dopa. The dopamine was then removed with 2 × 2 ml of 10 M HOAc and the radioactivity of each 2 ml aliquot measured in 10 ml of scintillation fluid (Packard Instagel) [29]. In all latex prepns only the complete assay mixture gave radioactivity in the isolated dopamine when L-dopa D.C. was present. Boiled prepn had no radioactivity in the isolated dopamine. *PC*: on Whatman No. 1 paper in ProH–H_2O–*n*-BuOH (5:7:10).

(b) *Determination of activity by measurement of* $^{14}CO_2$ *evolution from* L-*dopa*-1-[^{14}C]. A manometric modification of the method of ref. [30] was used. Warburg flasks with two side arms were used. The central well contained corrugated filter paper soaked in ethanolamine–methoxyethanol, 2:1 (0.2 ml). The main compartment contained 500 mM mannitol 100 mM phosphate buffer pH 7.2 to a total vol. of 1 ml; latex prepn (0.1 ml), DIECA 10 mM; and ascorbate 20 mM. The substrate 0.2 mM (0.2 ml) was placed in one side arm and 2N H_2SO_4 (0.2 ml) was placed in the second side arm. The latex prepns were pre-incubated with DIECA and ascorbate for 10 min at the bath temp. The reaction was initiated by the addition of the substrate and incubation was at 35° for 10 min. The reaction was stopped with 2N H_2SO_4 and after a further 10 min the reaction flasks were disconnected and the ethanolamine impregnated filter paper placed in a vial with scintillation fluid for counting the $^{14}CO_2$ liberated during the reaction. Controls contained boiled latex prepn.

Protein determinations. The protein content of all latex preps was determined using the method of ref. [31].

REFERENCES

1. Spenser, I. D. (1966) *Lloydia* **29**, 71.
2. Wilson, M. L. and Coscia, C. J (1975) *J. Am. Chem. Soc.* **97**, 431.

3. Battersby, A. R., Jones, R. C. F. and Kazlanskas, R. (1975) *Tetrahedron Letters*, 1876.
4. Jindra, A. (1967) *Acta Facult. Pharm. Bohenoslov.* B, 7.
5. Asghar, S. S. and Siddiqi, M. (1970) *Enzymologia* **39**, 289.
6. Udenfriend, S., Lovenberg, W. and Sjoerdsma, A. (1959) *Arch. Biochem. Biophys.* **85**, 487.
7. Tocher, R. D. and Craigie, J. S. (1966) *Can. J. Botany* **44**, 605.
8. Schmalfuss, H. and Heider, A. (1931) *Biochem. Z.* **236**, 226.
9. Tocher, R. D. and Tocher, C. S. (1972) *Phytochemistry* **11**, 1661.
10. Fairbairn, J. W. and Djote, M. (1970) *Phytochemistry* **9**, 739.
11. Fairbairn, J. W., Hakim, F. and Kheir, Y. (1974) *Phytochemistry* **13**, 1133.
12. Antoun, M. D. and Roberts, M. F. (1975) *Planta Med.* **28**, 6.
13. Antoun, M. D. and Roberts, M. F. (1976) Communication to the American Society of Pharmacognosy—Memphis 1976.
14. Mason, H. A. (1955) in *Adv. Enzymol.* **16** (Nord, F. F. ed.). p. 105. Wiley, New York.
15. Roberts, M. F. (1971) *Phytochemistry* **10**, 3021.
16. Davison, A. N. (1956) *Biochim. Biophys. Acta* **19**, 131.
17. Christenson, J. G., Dairman, W. and Udenfriend, S. (1970) *Arch. Biochem. Biophys.* **141**, 356.
18. Brown, B. R. (1967) in *Oxidative Coupling of Phenols* (Taylor, W. I. and Battersby, A. R. eds.) p. 167. Marcel Dekker.
19. Tran, N. and Laplante, M. (1972) *Int. J. Biochem.* **3**, 117.
20. Fellman, J. H. and Roth, E. S. (1971) *Biochemistry* **10**, 408.
21. Schott, H. F. and Clark, W. G. (1952) *J. Biol. Chem.* **196**, 449.
22. Corgier, M. and Pacheco, H. (1975) *Biochimie* **57**, 1005.
23. Sourkes, T. L. (1966) *Pharmacol. Rev.* **18**, 53.
24. Lovenberg, W., Weissbach, H. and Udenfriend, S. (1962) *J. Biol. Chem* **237**, 89.
25. Awapara, J., Sandman, R. P. and Hanly, C. (1962) *Arch. Biochem. Biophys.* **98**, 520.
26. Hagen, P. (1962) *Brit. J. Pharmacol.* **18**, 175.
27. Coulson, W. F., Henson, G. and Jepson, J. B. (1968) *Biochim. Biophys. Acta* **156**, 135.
28. Roberts, M. F., unpublished results.
29. Masuoka, D. T., Drell, W., Scott, H. F., Alcaraz, A. F. and James, E. C. (1963) *Anal. Biochem.* **5**, 426.
30. Jeffay, J. and Alvarez, J. (1961) *Anal. Chem.* **33**, 612.
31. Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **19**, 265.