will be very difficult, if not impossible (see figure 3). The separation of the centres of the pictures must not exceed 65 mm, the average inter-eye distance in humans. Any picture separation larger than this will need divergence of the eye axes in order to fuse the frames; needless to say that, if it is possible at all, eye strain will result[16].

Conclusion. As viewing of stereoscopic pictures is very easy after some practice is gained, and gives a much better impression of steric relationships, much more use should be made of stereoscopic photographs of molecular models. Recently 2 colour photos of a Beevers model of cyclosporin A were reproduced in Helvetica Chimica Acta to show the conformation of the native peptide[17]. However, if instead of them 1 stereo pair of colour photos had been printed, a much better idea of the proposed conformation could have been obtained.

16 The trained observer may want to look at such an example; e.g. H. B. Bürgi, H. Gehrer, P. Strickler and F. K. Winkler, Helv. chim. Acta *59*, 2558 (1976), see p. 2560.

17 T. J. Petcher, H.-P. Weber and A. Rüegger, Helv. chim. Acta *59*, 1480 (1976), see p. 1488a.

PRO EXPERIMENTIS

A facile one-step synthesis of cysteinyldopas[1] using mushroom tyrosinase

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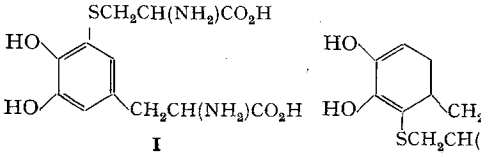
Summary. A convenient one-step procedure, based upon the tyrosinase co-oxidation of dopa and cysteine, is reported for the synthesis of 5-S-cysteinyldopa (**I**) in 74% yield. Secondary products of the reaction turned out to be 2-S-cysteinyldopa (**II**, 14%), 2,5-S,S-dicysteinyldopa (**IV**, 5%), and the hitherto unknown 6-S-cysteinyldopa (**III**, ~ 1%).

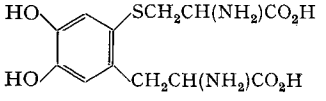
In the past few years, the unique catechol amino-acids, 5-S-cysteinyldopa (**I**) and 2-S-cysteinyldopa (**II**), have been the object of extensive investigations showing their central role in the biosynthesis of phaeomelanins including trichochromes[3,4]. More recently, a related compound, 2,5-S,S-dicysteinyldopa (**IV**), has been identified as the major constituent of the reflecting spheres in the eye of some fishes[5]. Increasing interest in these amino-acids is provided by the finding that large amounts of 5-S-cysteinyldopa and related metabolites are found in the urine of patients with malignant melanoma[6,7], while in healthy humans the level of excretion is very low. Accordingly, analysis of cysteinyldopas in the urine has been proposed as a method for the chemical diagnosis of melanoma metastates.

Although a chemical synthesis for 5-S-cysteinyldopa has been described[8], to facilitate studies in these fields we report here a simple and more convenient enzymic procedure which makes readily available all the cysteinyldopas including a new isomer[9], 6-S-cysteinyldopa (**III**).

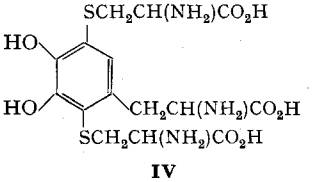


I

II



III



IV

Synthesis and isolation of cysteinyldopas (**I–IV**). After several trials, the optimal conditions for the preparation and separation of cysteinyldopas were established as follows: a solution of L-dopa (99 mg; 0.5 mmoles) and L-cysteine (121 mg; 1.0 mmole) in 0.05 M sodium phosphate buffer, pH 6.8 (60 ml) was vigorously stirred at 22 °C (oxygen not bubbled into the solution) in the presence of mushroom tyrosinase (18 mg; 2750 units/mg; from Sigma Chem. Co.) and the course of the reaction was followed by monitoring the UV spectrum (in 0.1 N HCl) of aliquots taken at suitable intervals. After 30–45 min, the initial absorption maximum of dopa at 280 nm was completely replaced by new maxima at 255 and 293 nm, corresponding to the cysteinyldopa chromophore. At this stage, the oxidation was stopped by acidification to pH 1 with 6 N HCl and the reaction mixture was passed through a column (1.8 × 12 cm) of Dowex 50 W-X 2 (200–400 mesh, H^+ form). After washing with 0.5 N HCl (250 ml), the column was eluted with 3 N HCl and fractions of 20 ml were collected and analyzed spectrophotometrically between 240 and 350 nm. Fractions 3–13 containing cysteinyldopas (**I–IV**) were combined together and evaporated to dryness to give a colourless residue which was taken up in 2 N HCl (2 ml) and chromatographed as described in the figure. 4 peak

1 The generic term 'cysteinyldopa' is proposed to designate the various cotechol amino-acids arising from addition of cysteine to dopaquinone.

2 This work was supported in part by a grant from Consiglio Nazionale delle Ricerche, Roma.

3 H. M. Thomson, Angew. Chem. Int. Ed. *13*, 305 (1974).

4 G. Prota and R. H. Thomson, Endeavour *35*, 32 (1976).

5 S. Ito and J. A. C. Nicol, Tetrahedron Lett. *1975*, 3287.

6 H. Rorsman, A-M. Rosengren and E. Rosengren, Yale J. Biol. Med. *46*, 516 (1973).

7 G. Agrup, P. Agrup, T. Anderson, B. Falck, J. A. Hausson, S. Jacobsson, H. Rorsman, A-M. Rosengren and E. Rosengren, Acta derm.-vener., Stockh. *55*, 337 (1975).

8 G. Prota, G. Scherillo and R. A. Nicolaus, Gazz. chim. ital. *98*, 495 (1968).

9 Evidence for this compound has been previously obtained by GLC-MS analysis (H. Rorsman and E. Rosengren, private communication).

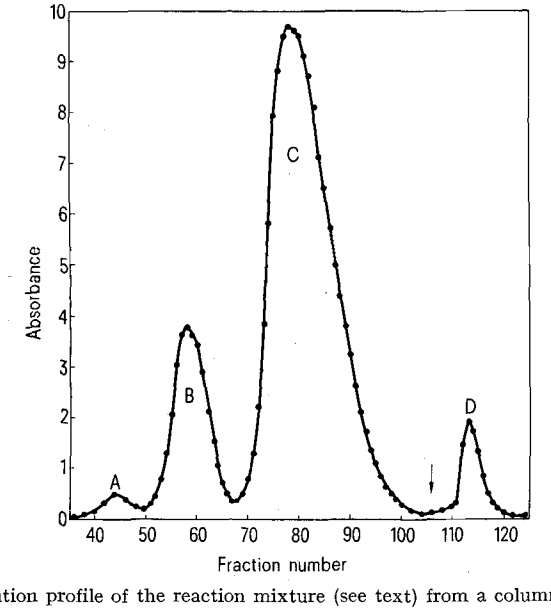
Chromatographic and spectral properties of cysteinyldopas

Peak in the figure (fractions)	Compound	Yield[a]	R_f[b]	Retention time[c] (min)	UV[d] λ_{max}	nm (ε)	λ_{min}	nm (ε)	PMR[e] δ
A (39–47)	6-S-Cysteinyldopa (**III**)	3 mg (≃1%)	0.57	51.5	292 255	(1.00) (1.91)	275 244	(0.62) (1.69)	6.93 (s), 7.21 (s)
B (52–66)	2-S-Cysteinyldopa (**II**)	30 mg (14%)	0.61	51.5	295 256	(3280) (2470)	272 250	(1220) (2400)	6.90, 7.03 (AB_q, J = 8 Hz)
C (70–98)	5-S-Cysteinyldopa (**I**)	154 mg (74%)	0.71	78.5	293 254	(2860) (3590)	273 248	(1360) (3420)	6.93, 7.01 (AB_q, J = 2 Hz)
D (105–119)	2,5-S,S-Dicysteinyldopa (**IV**)	20 mg (5%)	0.40	55	302 273	(3140) (8560)	297 251	(3090) (3710)	7.16 (s)

[a] Percent yields are spectrophotometrically determined on the basis of the molar extinction coefficients (ε). [b] Relative to dopa on a Merck cellulose plate with n-propanol-1N HCl (3:2, v/v). [c] On the long column (Beckman type M 72 resin) of a Beckman model 120 B amino acid analyzer using as the eluent the pH 4.25 buffer; dopa appeared at 66 min. [d] Taken in 0.1 N HCl; for **III** ε was not determined and therefore the values in parentheses are relative absorbances. [e] Taken in 1N DCl using t-butanol (δ 1.28) as an internal reference. Only the aromatic signals are reported which are indicative of the aromatic substitution patterns.

fractions (A–D) appeared from the column which were concentrated in a rotary evaporator and then dried in vacuo over P_2O_5 and NaOH.

TLC of fractions B–D gave single spots detectable by UV-light or ninhydrin and $FeCl_3$ reagents, while fraction A contained 2 compounds which were separated by paper chromatography on Whatman 3 MM in n-propanol-1 N HCl (2:1, v/v). Elution of the major and faster moving band with 0.1 N HCl gave pure 6-S-cysteinyldopa (**III**). Although the cysteinyldopas obtained as above were practically homogeneous and almost colourless, for analytical purposes they were further purified by re-chromatography on a small column of Dowex 50 W (eluent: 2 N HCl). After this step, compounds **I** and **II** could also be crystallized as needles by evaporation of the eluates and subsequent treatment of the residues with a few drops of 6 N HCl.



Elution profile of the reaction mixture (see text) from a column of Dowex 50 W–X 2 (200–400 mesh, H^+ form) showing the clear-cut separation of the cysteinyldopas formed. The column (1.8 × 20 cm) was eluted with 2N HCl at a flow rate of 50 ml/h; fractions of 8 ml were collected and monitored at 293 nm for **I–III** (until fraction 105) and at 302 nm for **IV** (from fraction 106; indicated by the arrow). After this latter compound had started to emerge, elution was carried on with 4 N HCl.

Results and discussion. Biogenetically, 5-S-cysteinyldopa (**I**) and 2-S-cysteinyldopa (**II**) arise from the 1,6-addition of cysteine to dopaquinone produced by tyrosinase oxidation of dopa[3,4]. Although this enzymic reaction has been studied in vitro in various laboratories[5,10–12], it has never been used for preparative purposes[13], mainly because of the lack of adequate methods for separating the cysteinyldopas formed. The results described here show that: a) under suitable conditions, the reaction is practically quantitative giving mainly 5-S-cysteinyldopa (**I**) along with significant amounts of (**II**), (**III**), and (**IV**); b) the separation of the products can be readily accomplished by column chromatography on Dowex 50 W using as the eluent 2 N HCl.

The table summarizes the identification of the isolated cysteinyldopas and their chromatographic and spectral data. The assignment of structure **III** to the new cysteinyldopa isomer from peak A (see figure) is derived from the close similarity of its UV spectrum to those of **I** and **II**, and the PMR spectrum characterized by two 1H-singlets at δ 6.93 and 7.21, consistent with the presence of para-oriented aromatic protons. Apart from the preparative value, the present work made it possible to reexamine the positional reactivity of dopaquinone toward cysteine: the ratio of 5-, 2- and 6-S-cysteinyldopa being approximately[14] 74:14:1, which is very likely to be similar to their ratio of formation in phaeomelanogenesis[4].

10 S. Bouchilloux and A. Kodja, Bull. Soc. Chim. Biol. *42*, 69 (1960).
11 E. Fattorusso, L. Minale, S. De Stefano, G. Cimino and R. A. Nicolaus, Gazz. chim. ital. *99*, 969 (1969).
12 H. Rorsman, A-M. Rosengren and E. Rosengren, Acta derm.-vener., Stockh. *52*, 353 (1972).
13 A procedure has been described by Ito and Nicol[5] for the isolation of 2,5-S,S-dicysteinyldopa in 8.5% yield.
14 The ratio does not take into account the amount of 2,5-S,S-dicysteinyldopa formed.