treatments were only partially successful at inducing eventual ecdysis, animals which did not respond with premolt development to stage D_1 were given a second treatment of the same amount. The results show (table 1) that in the summer, the lobsters responded to the acetates as they had in winter[4] and that the principal acetate of the mixture produced the same response as the crude acetate mixture. All deaths indicated in tables 1 and 2 were of lobsters in late premolt (approximately at ecdysis). Our crystalline acetate melted at 147 °C, which agreed with one published melting point for ecdysterone triacetate (EAc_3)[5], but we were unable to get agreement with another[6]. However, we have concluded that our crystals were ecdysterone triacetate since 100 MHz NMR clearly showed the presence of only 3 acetate methyl groups with the rest of the spectrum like that of ecdysterone.

Table 2. Treatment of male and female lobsters with graded doses of ecdysterone triacetate

Treatment* (μg EAc_3/g)	Number** of lobsters	molts	deaths	Average time from last treatment*** to molt	death
Experiment 1, males					
Em (control)	10	0	0		
0.60/Em	10	1	0	44	
1.3/Em	10	2	0	47 ± 12	
2.5/Em	10	7	0	38 ± 8.1	
4.0/Em	10	7	3	30 ± 2.6	20 ± 11
5.0/Em	10	6	4	32 ± 6.4	29 ± 6.5
Experiment 2, females					
Em (control)	8	0	0		
0.6/Em	8	1	0	64	
1.3/Em	8	1	0	62	
2.5/Em	8	5	0	51 ± 1.1	
4.0/Em	8	7	0	32 ± 3.8	
5.0/Em	8	8	0	36 ± 8.4	
Experiment 3, males					
EtOH (control)	5	0	0		
0.6/EtOH	5	1	0	63	
1.3/EtOH	5	3	0	48 ± 15	
2.5/EtOH	5	4	0	31 ± 3.4	

*Em, FIA emulsion; EtOH, solution in 95% ethanol. **All lobsters were intermolt or early premolt (stage C to D_0). Groups were selected by random numbers. ***Second treatment was 70 days after first.

The results of dose-response relationship for EAc_3 in winter caught lobsters are shown in table 2. Early premolt (C–D_0 or D_0)[4] male and female lobsters were selected. Treatments were made in the same manner as previously (and above) but with a greater dose range. Again the first treatment had little visible effect and was therefore followed by a second of the same size. The results (see table 2) show an abrupt change from an effective dose at 2.5 μg/g to a largely ineffective one at 1.3 in a manner reminiscent of results with ecdysterone[2] but unlike them in that EAc_3-treated animals molted. Male and female lobsters responded alike. Little normal premolt development occurred in the controls, therefore the ecdyses observed were clearly forced by the treatment.

While emulsions were used in our first successful treatments[4] they were undesirable as they would not allow precision in injected doses. We therefore conducted an experiment (table 2) to test for the effectiveness of intramuscular injections of EAc_3 in ethanol (0.1 μl/g). Again, a second treatment was required to induce ecdysis. It would appear that the EAc_3 was at least as effective given this way as in FIA emulsion (table 2) and probably somewhat more effective due to more accurate dosage. We conclude that at the correct dosage, pure EAc_3 can force lobsters into what appear to be normal molts, and that the emulsions we originally used were not necessary. Overdoses of EAc_3 induce lethal premolt development like ecdysterone (table 1). Since the difference in effectiveness between ecdysterone and its triacetate is so clear cut with the lobster, a re-examination of the development induced by ecdysterone in other crustaceans and insects should be done but with careful dose-response determinations.

5 S. Imai, T. Toyosato, M. Sakai, Y. Sato, S. Fujioka, E. Murata and M. Goto, Chem. pharm. Bull. *17*, 340 (1969).
6 M. N. Galbraith and D. H. S. Horn, Aust. J. Chem. *22*, 1045 (1969).

COGITATIONES

How to view stereoscopic pictures of crystal structures and molecular models

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Summary. Methods are presented for practicing the viewing of stereoscopic pictures of crystal structures and molecular models without optical aids.

Our world, including chemistry, is three-dimensional. Thus, any two-dimensional representation of crystal structures, molecular models and the like in books or journals is a sort of compromise. Perspective drawings for instance, can evoke the feeling of space and shape only to a certain extent. There exist, however, several procedures for the printing of stereoscopic pictures. These pictures, when properly viewed, give realistic three-dimensional impressions.

The Xograph technique was used to illustrate enzyme-substrate interaction in the case of lysozyme[1]. This procedure gives beautiful colour pictures which show full three-dimensionality with no need for optical devices or special viewing skills. However, Xographs cannot be printed on regular pages as they have to be laminated with a plastic viewing screen. Accordingly the production costs are prohibitively high.

A. Meister et al. published stereophotographs of CPK models of α-aminoadipic acid enantiomers and analogs in connection with a study on the substrate specificity of sheep brain glutamine synthetase[2]. The pictures were printed in such a way that a three-dimensional view of the models could be seen with the aid of a mirror.

1 R. A. Harte and J. A. Rupley, J. biol. Chem. *243*, 1663 (1968).
2 V. P. Wellner, M. Zoukis and A. Meister, Biochemistry *5*, 3509 (1966); H. M. Kagan and A. Meister, Biochemistry *5*, 2423 (1966); Biochemistry *5*, 725 (1966).

Another possibility is to print the 2 frames which are to be seen by the right and left eyes, respectively, on top of each other, but in 2 different colours, typically red and green. They have to be viewed through correspondingly coloured glasses to produce the stereoscopic image. This technique was used in an inorganic chemistry textbook to illustrate the tetrahedrally substituted carbon atom, molecular structures, optical isomerism and crystal lattices[3].

The most simple method of reproduction, however, is to print the 2 frames side by side. This is called a stereoscopic pair view of the crystal structure or molecular model in question. Such stereo pairs often are computer-generated from crystallographic data by means of the ORTEP program[4]. Or they can be obtained when photographs of an object are taken from 2 slightly different camera positions.

ORTEP pairs are widely used for the representation of crystal and molecular structures and unit cell packings. Volumes 58 and 59 of Helvetica Chimica Acta presented more than 40 such pairs, whereas in volume 98 of the Journal of the American Chemical Society even far more than 100 were published. In a similar way computer-generated stereo pairs of protein structures have been printed in journals[5] as well as in textbooks[6]. A stereo picture even appeared in an advertisement for a general chemistry text[7].

Stereoscopic photographs of molecular models and other objects have, on the other hand, been published only sparingly. Electron density map and Kendrew models of ribonuclease S were shown for instance by Wyckoff et al.[8], and stereo photos of Dreiding models of oligonucleotide helices appeared in a paper from our laboratory[9]. Stereoscopic pair views, which are as easily prepared as they can be viewed, are far superior to any kind of perspective drawing, as soon as only moderately complex stereochemical relationships are to be shown. Figure 1 presents a perspective drawing of a polycyclic molecule obtained from an intramolecular cycloaddition of a binaphthyl compound[10]. One has to study this drawing carefully to make out the overall stereochemistry of the molecule, which can be perceived at the first glance from the corresponding stereoscopic pair (figure 1).

Viewing stereo pairs without optical aid. One can look at stereoscopic pairs using a viewer much like using a stereo slide viewer. But with some practice, stereo pairs can easily be viewed without any optical devices. Many chemists are not aware of this and simply disregard stereo pairs which they encounter in the literature. Therefore some hints are given below for the correct viewing of stereo pictures.

The pictures should be well and evenly illuminated and should lie flat, and not be slightly convex as is often the case when a journal or book is opened. The 2 frames of the pair must be properly aligned and the axis through the centre of the 2 pictures must be made parallel to the axis through the observer's two eyes. If these rules are not followed, it will be impossible to fuse the 2 frames properly. The eyes are then relaxed, which will cause them to lose convergence and look into infinity. This will produce the impression of 4 unsharp pictures. As soon as the 2 inner pictures are superimposed, they will fuse to give a three-dimensional image, which, however, is still unsharp, as the relaxed eyes are focused to infinity. The stereo picture must then be brought into focus without the fused frames becoming separated again. When properly done, the 2 printed frames will now appear as 3 sharp pictures with the centre one being three-dimensional[11]. A trained observer may now scan this three-dimensional picture just as he would scan real molecular models to look at different details.

The main problem for the untrained observer arises from the fact that for the fusion of the 2 frames of a stereo pair into a three-dimensional image, the left eye has to look at the left picture while the right eye has to look at the right one. Since the 2 frames are printed side by side, the axes of the eyes are nearly parallel under these circumstances, which is normally only the case when one looks

3 A. F. Hollemann and E. Wiberg, Lehrbuch der Anorganischen Chemie, Suppl. Molekül- und Gitterstrukturen in stereoskopischer Darstellung. Walter de Gruyter & Co., Berlin 1964/1963.

4 C. K. Johnson, ORTEP, Report ORNL-3794 revised, Oak Ridge National Laboratory, Oak Ridge, Tenn. 1965; see also D. Y. Curtin, J. chem. Educ. *50*, 775 (1973).

5 W. N. Lipscomb, Accts. chem. Res. *3*, 81 (1970).

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7 J. chem. Educ. *52*, A47 (1975).

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9 U. Séquin, Experientia *29*, 1059 (1973).

10 Y. Nakamura, R. Hollenstein, J. Zsindely, H. Schmid and W. E. Oberhänsli, Helv. chim. Acta *58*, 1949 (1975).

11 This impression of 3 pictures can be vexing at first. A piece of cardboard may be inserted between the 2 printed frames and extended towards the observer's nose. Then the 2 frames will fuse into only 1 picture, the stereoscopic one. As soon as some practice is gained, the cardboard can be omitted. Cf. M. M. Crozat and S. F. Watkins, J. chem. Educ. *50*, 374 (1973).

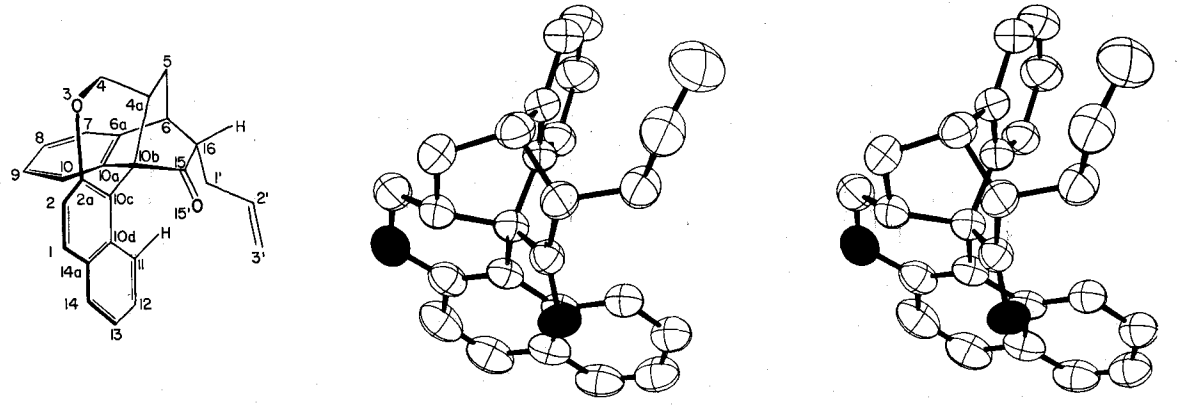

Fig. 1. Perspective drawing and stereo pair view of a polycyclic molecule. Note that the 2 representations are oriented in different ways (reprinted from Helv. chim. Acta[10] with permission).

into infinity. However, in order to see a sharp stereoscopic picture, one has to focus the eyes at the same time to about 25 cm, the normal reading distance. This – parallel eye axes and at the same time focus at 25 cm – is an uncommon situation for the eyes and therefore has to be practiced[12].

For training purposes, the discrepancy between convergence and focus of the eyes may be alleviated by 2 methods:

a) The separation of the 2 frames of a stereo pair can be made smaller, best by making a high quality photocopy, cutting the pictures out and remounting them in proper alignment (be sure not to interchange the left and right pictures). The smaller separation will now allow the eye axes to converge to some extent, which will facilitate the fusing of the 2 frames. As one gets more practice, the separation can be gradually increased until the normal separation of 60–65 mm can be handled. The example given in figure 2 is especially well suited for this purpose, being a rather slender molecule[13].

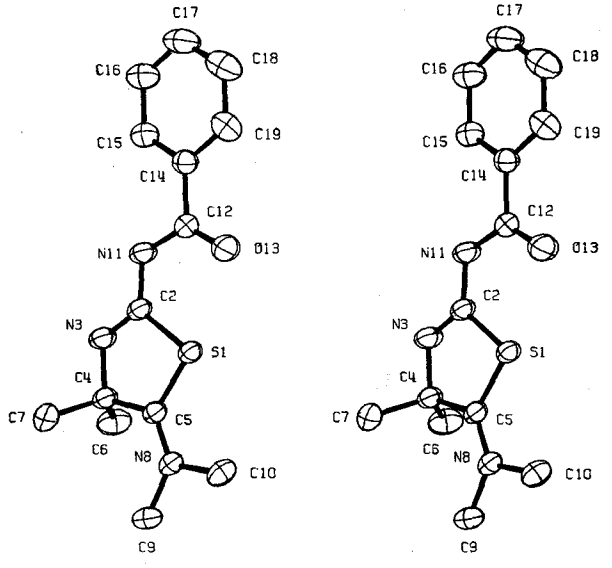

Fig. 2. Simple stereo pair view, suitable for practicing (reprinted from Helv. chim. Acta [13] with permission).

b) The other possibility is to keep the observing distance at the beginning rather large, say 1–2 m. The pictures are best leaned against or taped to a window. Then the eyes are aimed over the top rim of the stereo pair and through the window at a distant object. The stereo frames will then fuse almost with no effort and only a slight focus shift (from infinity to 1–2 m) is necessary to obtain a sharp three-dimensional vision. As practice is gained, the viewing distance may be gradually decreased down to the normal reading distance of 25–30 cm.

With all the methods in which optical devices are necessary for the viewing of stereo pictures, these devices somehow eliminate the discrepancy between convergence and focus of the eyes. In the Xograph and red/green techniques, the 2 stereo frames are printed superimposed, thus allowing normal convergence of the eyes. However, a viewing screen or coloured glasses are necessary to make sure that each eye sees only the 1 frame which it should. The mirror technique also allows normal eye convergence by optically superimposing the 2 frames. When using a stereo viewer, on the other hand, one can leave the eyes aimed and focused at infinity while the focus shift from infinity to the viewing distance (with most viewers between 5 and 15 cm) is accomplished by the stereo viewer's lenses.

Making stereo photographs. Stereo photographs may be taken along the guidelines given by McGrew for making stereoscopic slides[14]. The procedure consists essentially of taking 2 pictures from 2 different camera positions. The separation between these is, in contrast to slide making, not critical when preparing photographs to be reproduced in print. Any distortions in depth, that might arise from the use of a separation other than optimal, are negligible and hardly noticeable. In the author's laboratory, 65 mm have proved to give satisfactory results.

On the printed page, the 2 photographs making a stereo pair must be reproduced at exactly the same magnification[15] and be properly aligned. If the 2 frames are misaligned, their fusing into a three-dimensional image

12 Practicing should not be overdone; eye strain may result.

13 U. Schmid, H. Heimgartner, H. Schmid, P. Schönholzer, H. Link and K. Bernauer, Helv. chim. Acta *58*, 2222 (1975).

14 L. A. McGrew, J. chem. Educ. *48*, 531 (1971).

15 The effect produced by different magnification ratios can be seen in K. Jonas, D. J. Brauer, C. Krüger, P. J. Roberts and Y.-H. Tsay, J. Am. chem. Soc. *98*, 74 (1976), see p. 79.

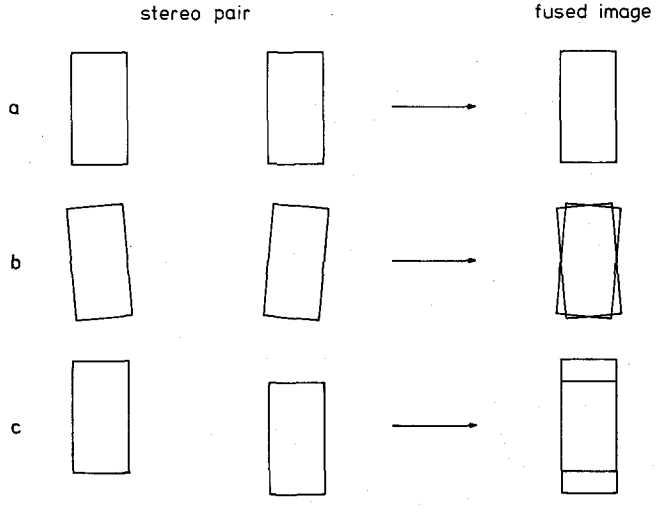

Fig. 3. Alignment of the 2 frames of a stereo pair. a) Correct alignment. b) Tilt. c) Vertical displacement; this can be corrected to some extent by making the axis through the two eyes parallel to the axis through the centres of the 2 frames.

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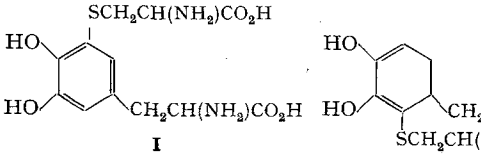
Summary. A convenient one-step procedure, based upon the tyrosinase co-oxidation of dopa and cysteine, is reported for the synthesis of 5-S-cysteinyldopa (**I**) in 74% yield. Secondary products of the reaction turned out to be 2-S-cysteinyldopa (**II**, 14%), 2,5-S,S-dicysteinyldopa (**IV**, 5%), and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

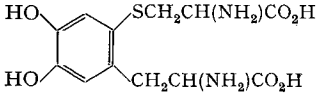
In the past few years, the unique catechol amino-acids, 5-S-cysteinyldopa (**I**) and 2-S-cysteinyldopa (**II**), have been the object of extensive investigations showing their central role in the biosynthesis of phaeomelanins including trichochromes[3,4]. More recently, a related compound, 2,5-S,S-dicysteinyldopa (**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**).

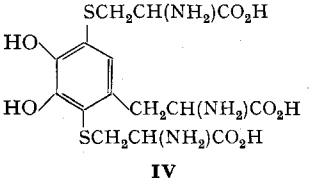


I

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.

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