

## Automatic Crystal Alignment and Intensity Data Collection with a Computer Controlled Diffractometer

BY RICHARD L. VANDLEN AND A. TULINSKY

*Departments of Chemistry and Biochemistry, Michigan State University, East Lansing, Michigan 48823, U.S.A.*

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A program for a computer controlled X-ray diffractometer is described that can compensate for crystal motion. In addition, a scheme particularly suitable for protein intensity data collection is outlined. The crystal aligning subroutine operates on the principle that if the crystal is found to have moved, a new orientation can be found for it with respect to the diffractometer coordinate system. The ensuring intensity data collection routine then bases its subsequent calculations on the new orientation. The intensity data collection routine is based upon a 'wandering' count-six-drop-two step scan procedure. An example and some experiences with the system are discussed and some protein intensity measurements are compared.

### Introduction

Automated X-ray diffractometers, particularly those under computer control, are currently prominently in vogue as a means of measuring X-ray intensity data for structure determination. The complexity of the structures examined with such instruments ranges from the relatively simple, where the diffractometer serves more as a convenience, to biological macromolecules like globular proteins and enzymes, where the diffractometer practically proves to be a necessity. These computer controlled systems are flexible by virtue of their program control, they operate reasonably fast and efficiently and in principle, they can be entrusted to perform unattended and to gather useful data, observations and other such information continuously. However, the independence of the computer controlled diffractometer rests upon several assumptions, the foremost of which is of concern to us here: that the crystal remains in the same position without changing its orientation throughout the course of intensity data collection. Specimens mounted on a glass fiber with adhesive generally satisfy this assumption; on the other hand, a protein crystal mounted in contact with its mother liquor (in a capillary adhering by surface tension interaction to the wall), in general, is continuously reorienting during the course of an intensity data collection. Thus, some type of manual intervention is required when the crystal misalignment becomes severe enough to affect the intensity measurements adversely and in this manner, the independence of the system is imposed upon. Needless to say, the intervention can be inconvenient at times and always time consuming compared with computer capabilities.

One way to cope with the problem is to program the computer to monitor the alignment of the crystal during data collection and to react appropriately when the crystal has been found to have moved. We have developed such a software package and have been employing it with our diffractometer system for over a

year (Picker 4-circle FACS-I with a DEC 4K PDP-8 and a DEC 32K DF32 disc). We would now like to present some details concerning our system and some experiences we have encountered in its operation. The software to be described is based upon Picker's original program package (*FACS-I Program Manual*, 1968).

### General

A severe limitation of our original version of a PDP-8 computer controlled diffractometer was associated with the computer itself in that it operated with only a 4K bank of magnetic core memory. Since our computer is not equipped with the extended arithmetic option or floating point hardware, half of magnetic core is devoted to routines associated with arithmetic and input-output operations. Consequently, the flexibility concomitant with programming was infringed upon by the limitations of the memory of the computer. Furthermore, the latter caused the system to require excessive paper tape inputting in order to change from one phase of operation to another.

These difficulties and inconveniences were compounded in our case by crystal motion (protein), crystals that were essentially moving continuously during the course of intensity data collection. The problem was solved by programming the computer to track the movement of the crystal\* and to react in a suitable fashion; in order to accomplish this, the memory of the computer was expanded by adding a 32K magnetic disc file from which an entire core memory load can be read in about 600 milliseconds. In this way, the system achieved more nearly true independence in that,

\* Assumed to be translationally stationary. The validity of the assumption is intimately connected with the size of the X-ray source, the size of the source collimator and detector apertures and the size of the crystal. As long as all the former are somewhat larger than the crystal, small translations can be tolerated without introducing any large effects in the angular measurements.

once control was transferred to the computer, manual intervention was no longer required for motion of a crystal. On the other hand, various built-in program stops included in the software terminated data collection for a variety of reasons (primarily recognition of catastrophic situations).

Concurrent with building an automatic crystal aligning software, a resident disc monitor was written and an intensity data collection package was rewritten to correspond more nearly to our specific needs (protein intensity data collection). The latter basically consisted of introducing a count-six-drop-two  $\omega$  step scan procedure (Wyckoff, Doscher, Tsernoglou, Inagami, Johnson, Hardman, Allewell, Kelly & Richards, 1967). Here the intensity is measured at each of six steps of the most sensitive angular position ( $\omega$ ) and the four largest measurements are used to form the intensity of the reflection. At the same time, we entered all of our separate subroutines onto the disc as system programs and in this way bypassed tape paper inputting to the computer.

#### Automatic crystal aligning subroutine

The automatic crystal aligning subroutine operates on the principle that if the crystal is found to have moved, a new orientation can be found for it with respect to the diffractometer coordinate system. The ensuing data collection routine then bases its subsequent calculations with respect to the new orientation.

Motion of the crystal is conveniently detected by comparing measured monitored intensities with pre-

designated values of these intensities. The monitored reflections are chosen such that their intensities are very sensitive to small angular displacements and in such a way as to assure that they completely define the orientation of the crystal. Our protein crystals are always mounted with the monoclinic unique axis ( $b$ ) of the crystal parallel to the azimuthal axis of a four-circle system, so we employ the 0, 18, 0 reflection ( $2\theta = 23.8^\circ$ ) over the  $a$  and the  $c$  axes (about  $78^\circ$  apart) to define the coincidence of the crystal  $b$  axis and the  $\varphi$  axis of the diffractometer. The intensities of this reflection are extremely sensitive to small displacements in  $\omega$  ( $0.05^\circ$  corresponds to about a 10% decrease). Monitoring two positions  $78^\circ$  apart in  $\varphi$  essentially corresponds to monitoring each arc of the goniometer head. Another reflection in general reciprocal space (non-special angles) is used to monitor the angular displacement in  $\varphi$  in the event there is no angular displacement at  $\chi = 90^\circ$  (twisting about the  $b$  axis occurs more frequently than originally anticipated). These standard reflections are measured periodically (at approximately one hour intervals) and if the intensity of any one of them decreases below some predesignated value, ( $0.9 \times$  their initial intensity), the data collection routine transfers control to the crystal aligning subroutine.

The crystal aligning subroutine determines the angular coordinates of selected reflections by centering their intensities in  $\omega$ ,  $\chi$  and  $2\theta$ . The various angles are obtained by seeking maximum intensity as inferred from measurements locating the points of  $0.9 \times$  maximum intensity. Usually eight reflections, well distributed in reciprocal space and containing some high

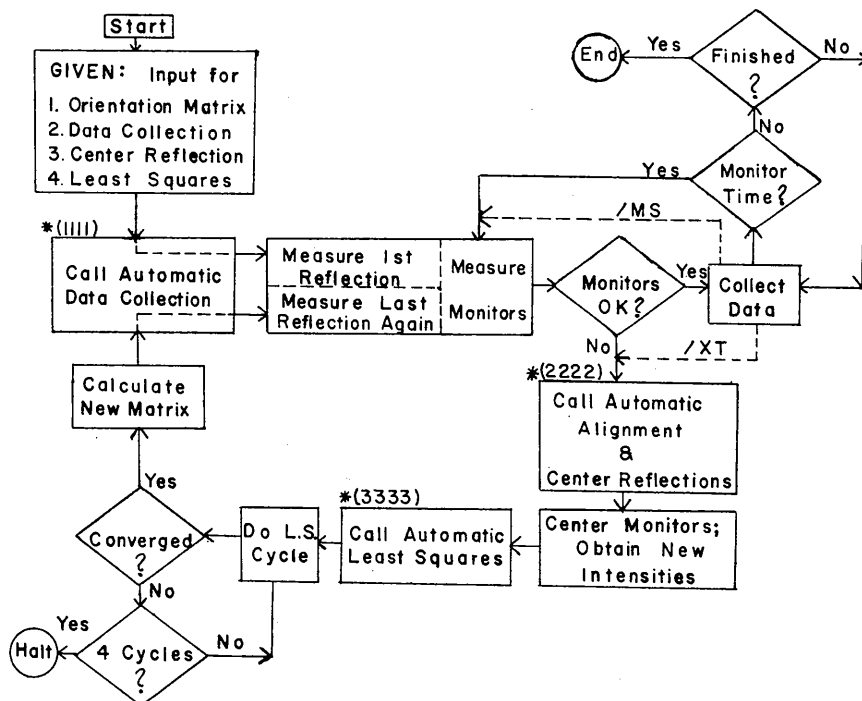


Fig. 1. Flow chart of the automatic crystal alignment and intensity data collection program.

$\chi$  valued members ( $\chi \sim 90^\circ$ ), are employed for this purpose.\* During the time spent in this subroutine, new angular coordinates are also obtained for the monitored reflections and the previously predesignated initial intensities of the monitors are replaced with new intensity measurements at the new peak positions. We require such a procedure since the intensities of the standard reflections decrease as a function of X-ray exposure to the crystal (proteins). If the standard intensities decay 10% due to X-ray exposure in the absence of crystal misalignment, the program does not differentiate the cases and proceeds with a realignment. We have had occasion to observe such behavior with our protein crystals. The interval between successive realignments simply due to decay is about 10–12 hours.

When remeasurement of the angular positions of the preselected reflections is completed, a least-squares orientation routine is entered into core memory from the disc and a best least-squares fit of the observations is obtained to the cell parameters of the crystal and the orientation of the crystal with respect to the diffractometer coordinate system. When convergence is attained (parameter shifts less than a specified amount, say  $<0.01^\circ$  and  $0.01 \text{ \AA}$ ), another cycle is performed, the observed and calculated values are printed and a new orientation matrix is calculated to be used in subsequent work by the intensity data collection routine. Transfer then occurs to the latter routine. The time expended by the crystal alignment subroutine in the form described above is approximately 30 minutes (about 3 minutes per aligning reflection).

#### Intensity data collection routine

Since the criteria we employ to detect crystal misalignment (outlined above) are sufficiently stringent to preclude any discernible intensity measurement errors due to misalignment in the previous hour of data collection, the intensity data collection routine begins by remeasuring the last measured intensity, followed by the measurement of the monitored reflections and proceeding from there. Of course, the previous hour of data collection could have been affected by what we designate as a catastrophic situation, such as an inordinately severe misalignment, and to this extent some of the intensity measurements might be in serious error. However, some such situations are tested for in the crystal aligning subroutine and all ultimately lead to programmed halts. Otherwise, they generally appear as fairly obvious (and have been observed on several occasions in our protein work and originated from different causes: crystal flipping in orientation by about  $90^\circ$ ; bursting of the mother liquor droplet securing the

\* The most sensitive angle in our system is  $\omega$ . Small errors in  $\chi$  of small  $\chi$ -valued reflections ( $\chi \sim 0^\circ$ ) produce very large errors in  $\omega$  at  $\chi \sim 90^\circ$ . Although errors in  $\omega$  produce errors in  $\chi$ , (1) the error in  $\omega$  is always much smaller than in  $\chi$  and (2) the  $\chi$  dependence is broad and relatively insensitive. Our procedure simply assures a more reliable orientation matrix.

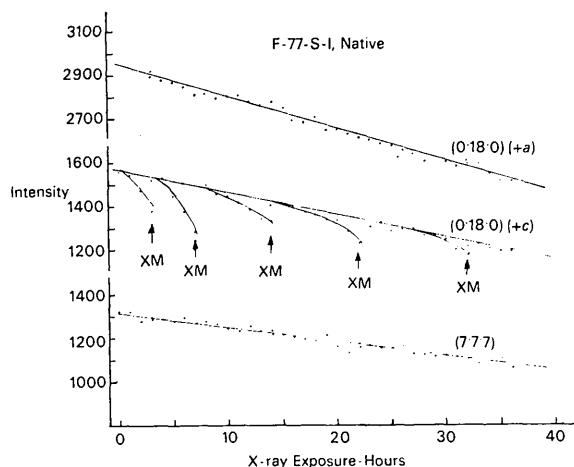


Fig. 2. Behavior of monitored reflections during an intensity data collection.

crystal mount with a resultant unrecoverable misalignment; filter wheel binding between filter positions ( $\alpha, \beta$ ) to eclipse the scintillation counter and produce zero intensity readings). Notwithstanding such unpredictable occurrences, the system was always able to recover more or less reasonably in that in most of these cases it refrained from proceeding in an unintelligent manner.

The data collecting routine is based on a step-scan procedure utilizing balanced filters (for  $\text{Cu } K\alpha$  in our case) to measure intensity and background. The step-scan is performed with respect to the most sensitive angular position ( $\omega$ ). The scan extends  $\pm 0.075^\circ$  from the calculated position and the scan is carried out in  $0.03^\circ$  increments. The intensity is measured for a preset time (4 seconds) at each of the steps and the four largest measurements are totalled to give the intensity of the reflection (count-six-drop-two; Wyckoff *et al.*, 1967). The crystal is then moved to the  $\omega$ -position displaying the highest intensity and background is recorded at this position (4 seconds) with a balanced Co filter. The total background is then calculated by multiplication of the raw background count by an appropriate constant. The  $0.15^\circ$  scan interval was chosen as a compromise among several competing effects with the most weight being given to the flat-top region of about  $0.1^\circ$  in  $\omega$  generally displayed by the reflections from our crystals under our operating conditions (take-off-angle, collimating apertures, *etc.*).

At times, the calculated  $\omega$  value ( $0.00^\circ$ ) does not correspond exactly to the observed value of the peak of a reflection, especially when the crystal has undergone some misalignment. To allow for such small fluctuations from the calculated  $\omega$  value, the step-scan program checks for the step of greatest intensity. If this step is at either end of the  $\omega$  step-range ( $-0.07$  or  $+0.08^\circ$ ), two additional steps are made in the reverse or forward direction respectively. If the step with maximum intensity is one step from either end,

then only one additional  $\omega$  step is made to  $-0.10$  or  $+0.11^\circ$ . The final reflection intensity is taken to be the sum of the four steps of highest intensity. The extended step-scan comes into effect only if the step with the highest intensity exceeds some minimum count; otherwise, the intensity of the reflection is considered to be too small to be pursued further. Since such a 'wandering' stepping procedure is not employed in measuring the intensities of the standard reflections, the procedure additionally assures the quality of the measurements between monitor times (larger misalignments of the crystal can be tolerated).

On the average, our system measures a reflection in about 30 seconds. Since our balanced filters are now operated by a solenoid system and the driving time to the next reflection is usually negligible (2–3 seconds, cell dimensions  $\sim 49 \times 67 \times 66 \text{ \AA}$ ) and our outputting station is internally buffered, the 30 seconds of each measurement are used very effectively. Thus, the system possesses the capability of measuring reasonably carefully 100–110 reflections per hour (some time is spent every hour measuring monitored reflections) or about 2500 reflections in a 24 hour period. This total

has been realized by us on a number of occasions when the crystal mount was exceptionally stable. Realignment in these cases occurred only once in the 24 hour period and then simply because a monitored reflection decreased by 10% in intensity due to exposure of the crystal to X-rays.

A flow chart of the program is shown in Fig. 1. The sections designated with asterisks are points where the program can be entered or interrupted. The console switch settings for a programmed halt at these points are given in parentheses. The latter permit the program to be interrupted in a convenient way (programmed halt) and continued in a logical manner. After the preliminary input information has been entered (Start), the program can be initiated or transferred to any of these sections with a retrieval of the section from disc memory. In addition, the program can be interrupted during Collect Data with the teletypewriter command /ID (Interrupt Data). Four options are then available: (1) (/MS) measure standards; (2) (/XT) transfer directly to alignment subroutine; (3) (/RD) resume Collect Data; (4) any of the previously discussed restart possibilities. The first two of these options are indicated in the flow chart as alternate routes (broken lines).

### An example and some results

Fig. 2 summarizes a typical behavior of monitored protein reflections during the course of an actual three-dimensional data collection. The solid straight lines drawn through the measured intensities approximate the intensity decrease suffered by the crystal with X-ray exposure. The symbols XM denote the monitor times at which the program decided that the crystal had moved and consequently, entered the realignment subroutine. Since XM was detected only on the 0, 18, 0 reflection over the  $c$  axis, it indicates that the crystal was reorienting at right angles to the  $c$  axis. This can be seen more clearly from Fig. 3, which summarizes the history of the movement of the crystal in terms of pertinent angles. Here, each point represents the angular positions of the reflections at XM times. Thus, it will be seen that the system realigns on a minimum angular displacement of about  $0.05$ – $0.07^\circ$  in  $\omega$ , which corresponds to a 10% decrease in intensity. Furthermore, from the overall behavior of the 0, 18, 0 reflection, it will be seen that it approximates theoretical expectations closely (displacement of  $\omega$  in one arc produces a corresponding displacement in  $\chi$  in the mutually perpendicular arc). In the present case, the crystal is reorienting much more in  $\omega$  at  $+c$  and this in turn produces a correspondingly large change in  $\chi$  at the  $+a, \varphi$  setting. Finally, from Fig. 2, it will be seen that the crystal was realigned at 4, 8, 14, 23, and 32 hours, suggesting that crystal movement was slowing down with time. Furthermore, upon realignment, the new monitored intensities agree well with their expected values (solid line).

Table 1 shows how the alignment of the crystal is

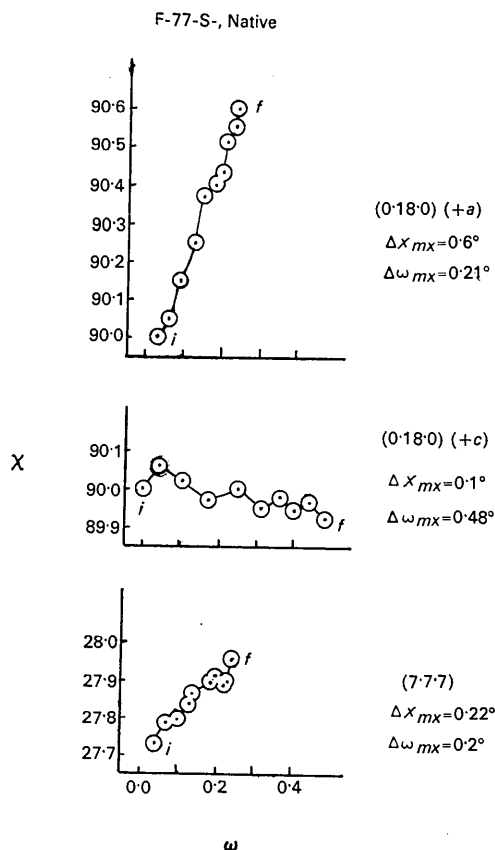


Fig. 3. History of the movement of a crystal in terms of pertinent angles of the monitored reflections;  $i$  and  $f$  are initial and final positions, respectively;  $mx$  denotes total angular displacement (55 hours X-ray exposure,  $\sim 70$  hours elapsed time). Each point represents the position of the crystal after realignment.

compensated for by the program. The reflections given in the Table are the primary and secondary orienting reflections (Busing & Levy, 1967), both of which have  $\chi$  values close to zero and a  $\Delta\theta$  of about  $90^\circ$  (for maximum sensitivity). The  $\chi_p$ ,  $\varphi_p$  and  $\chi_s$  parameters of these reflections fix the orientation of the crystal and these are varied in the least-squares procedure which employs eight aligning reflections (16 observations in  $\omega$  and  $\chi$ ; cell parameters are kept constant). The least-squares orientation of the crystal is given in the Calc. column. Inspection of the Obs. and Calc. columns will show that the program tracks the orientation of the crystal in a satisfactory manner.

Table 2 summarizes the kind of reproducibility in intensity measurements we have been obtaining with our overall system and particularly the 'wandering' step-scan procedure. The comparisons shown have been made with the enzyme  $\alpha$ -chymotrypsin or a derivative thereof. Some pertinent facts about the crystals are: (1) they are grown from about half-saturated ammonium sulphate at  $\text{pH} = 3.8\text{--}4.2$ ; (2) they are stored in 75% saturated ammonium sulphate,  $\text{pH} = 3.8\text{--}4.2$ ; (3) the crystals are monoclinic,  $a = 49.24 \pm 0.07$ ,  $b = 67.13 \pm 0.09$ ,  $c = 65.94 \pm 0.08$  Å,  $\beta = 101.77 \pm 0.07^\circ$  (average of five intensity data collection crystals), space group  $P2_1$ , with 4 molecules (M.W.  $\sim 25,500$ ) per unit cell (two per asymmetric unit).

In Table 2,  $N$  refers to the number of reflections used in a given comparison and  $R$  refers to a factor which is  $\frac{N}{\sum |F|_i - \langle |F| \rangle} / \sum \langle |F| \rangle$ , where  $|F|_i$  and  $\langle |F| \rangle$  are the

observed structure amplitudes of the reflections being compared and their average value, respectively. In these comparisons, all the reflections that were measured are included, irrespective of their intensities. Thus, not surprisingly, it can be seen from Table 2 that our best reproducibility obtains when the measurements are made on the same crystal ( $R \sim 0.02$ ). The reproducibility understandably deteriorates slightly ( $R \sim 0.03$ ) when the comparisons are extended to different crystals. The poorest reproducibility ( $R \sim 0.04$ ) comes with reflections that are remeasured after considerable exposure of a crystal to X-rays, particularly when the crystal is a heavy-atom derivative. In these cases, the fall-off in intensity with X-ray exposure is corrected for only in an average way (which we know is not strictly correct) so we attribute the poorer reproducibility primarily to this cause. An added complication enters into consideration of the heavy atom derivatives. Here, the net effect is a general broadening and loss of the diffraction pattern and it presents itself as an apparent partial loss of occupancy of the substituting electrons. Obviously, this can come about in a number of ways, a reasonable one being through differential changes in thermal parameters. Comparisons of 6 Å resolution ( $h0l$ ) data of heavy-atom derivatives before and after data collection ( $\sim 35$  hours X-ray exposure) usually gives an  $R$  value of about 0.05 on differences. This, in turn, corresponds to an overall change of about 10–20 electrons per asymmetric unit, which at times occurs mostly near one position, but which we feel can be tolerated.

Table 1. Observed and calculated positions of primary and secondary orienting reflections at several times during the data collection

Time	Orienting reflection	Initial		4 Hours		32 Hours		$\sigma$
		Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	
1200	$\omega_p$	0.02	0.0	0.01	0.0	0.02	0.0	$\pm 0.02$
	$\chi_p$	0.04	0.02	0.12	0.09	0.39	0.39	
	$\varphi_p$	173.84	173.82	173.82	173.81	173.82	173.81	
3021	$\omega_s$	-0.01	0.0	0.02	0.0	-0.02	0.0	$\pm 0.02$
	$\chi_s$	0.02	0.01	-0.09	-0.13	-0.22	-0.19	
	$\varphi_s$	84.49	84.50	84.50	84.48	84.47	84.49	

Table 2. Comparison of some protein intensity measurements

Crystal	$2\theta$ -range	$N$	$R$	Comments
Native-1	2.5—23.4°	242	0.022	166( $\pm hk0$ ) + 76( $hkl$ ) reflections measured on same crystal
Native-2	23.25—28.5	1009	—	1009 reflections from $h = -15$ to $h = -7$
Native-3	23.25—28.5	1009	0.032	2528 reflections from $h = -7$ to $h = +15$ ( $\sim 30$ hours X-ray exposure); followed by remeasurement of Native-2 data
Native-1, 2, 3	—	116	0.028	0.15° overlap in $2\theta$
NATIVE plus				
Pt <sub>1</sub> -1	2.5—20.0	197	0.018	197( $\pm hk0$ ) reflections measured on same crystal
Pt <sub>1</sub> -2	19.75—25.0	167	0.038	0.25° overlap in $2\theta$
Pt <sub>1</sub> -3	24.75—28.0	148	0.030	0.25° overlap in $2\theta$
NATIVE plus				
Pt <sub>2</sub> -1	2.5—22.5	407	—	407 reflections from $h = -12$ to $h = -2$
Pt <sub>2</sub> -2	2.5—22.5	407	0.043	2655 reflections from $h = -3$ to $h = +12$ ( $\sim 32$ hours X-ray exposure); followed by remeasurement of Pt <sub>2</sub> -1 data; 250 ( $hkl$ ) ( $h = -3$ only) + 157( $\pm hk0$ ) reflections

In view of the fact that our intensities are only approximately corrected for decay due to X-ray exposure and that the same applied to our absorption corrections (North, Phillips & Mathews, 1968), it is indeed remarkable that our reproducibility is so good. The comparisons made in Table 2 extend over eight different crystals and about 2300 reflections (~4600 measurements) and have an average  $R \sim 0.03$ . Preliminary indications from the data processing of some of our other derivatives show that a similar quality of agreement persists and thus, further reassures us of our procedures.

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## Structure Moléculaire et Cristalline du Bis(diméthyl-2,5-carboxylate-4-oxazolidine) de Cuivre Dihydraté en Relation avec la Synthèse de la DL-Thréonine

PAR GEORGES LARCHÈRES

*Laboratoire de Chimie-Physique, Centre Universitaire de Marseille-Luminy, 13 Marseille 8e, France*

ET MARCEL PIERROT

*Laboratoire des Mécanismes de la Croissance Cristalline, associé au C.N.R.S.,  
Faculté des Sciences, Sant Jérôme, 13-Marseille 13e, France*

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The structure of the copper(II) complex  $C_{12}H_{24}O_8N_2Cu$ , formed during the synthesis of DL-threonine, has been determined by X-ray diffraction. Unlike other determinations, the molecular structure found here is based on an oxazolidine. The structure is similar to that of the bis-prolinate of copper(II) dihydrate and this is confirmed by the infrared spectra.

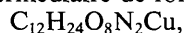
### Introduction

La thréonine (Shoemaker, Donohue, Schomaker & NH<sub>2</sub>

Corey, 1950), de formule  $H_3C-C^*H-C^*H-CO_2H$ , est  
|  
OH

un acide aminé indispensable à la vie; elle n'est pas synthétisée par l'organisme, aussi est-il nécessaire d'en ajouter aux aliments déficients en cet amino acide.

Parmi les méthodes de préparation de la thréonine (Asahi Chemical Industry Co. Ltd., 1965; Sato, Okawa & Akabori, 1957; Takaji, 1965; Maldonado, 1970), l'une des plus avantageuses est la suivante: formation d'un complexe intermédiaire de formule brute



obtenu par condensation, en solution aqueuse basique, de glycolle sur un excès d'acétaldéhyde, et en présence de carbonate basique de cuivre. La décomposition de ce complexe, par H<sub>2</sub>S, libère la thréonine.

L'intérêt de cette synthèse est d'orienter la réaction vers la formation d'une quantité prépondérante de l'isomère DL-thréonine, dans un rapport DL-thréonine/DL-allothréonine = 1,5.

Afin de trouver les conditions qui permettent d'augmenter ce rapport, il est important de connaître le mécanisme de ces réactions et pour cela, la structure du complexe  $C_{12}H_{24}O_8N_2Cu$  doit être connue.

Deux formules développées ont déjà été proposées pour ce complexe. Pour Sato, Takahashi, Imado, Sugimoto & Kotera (1961), le cuivre est lié aux fonctions acide et alcool de la thréonine, formant ainsi un complexe plan carré. Selon ces auteurs, l'acétaldéhyde a réagi sur la thréonine pour former une double liaison C=N, c'est-à-dire une base de Schiff. Belikov, Kuznetsova & Safonova (1967) proposent une formule dans laquelle le cuivre est hexacoordiné, formant 4 liaisons fortes avec la thréonine et 2 liaisons plus faibles avec 2 molécules d'acétaldéhyde. Ces formules ne sont pas satisfaisantes et en particulier, celle publiée par Sato et coll. est déduite d'une étude cristallographique