Acta Cryst. (1995). B51, 308–314

The Yellow Toxins Produced by *Cercospora Beticola*. V. Structure of Beticolins 2 and 4

BY THIERRY PRANGÉ* AND ALAIN NEUMAN

Chimie Bio-organique Structurale (URA 1430 du CNRS), UFR Biomédicale, 93012 Bobigny CEDEX, France

AND MARIE-LOUISE MILAT AND JEAN PIERRE BLEIN

Unité Associée de Phytopharmacie et Phytobiologie Cellulaire, INRA, Université de Bourgogne, BV 1540, 21034 Dijon CEDEX, France

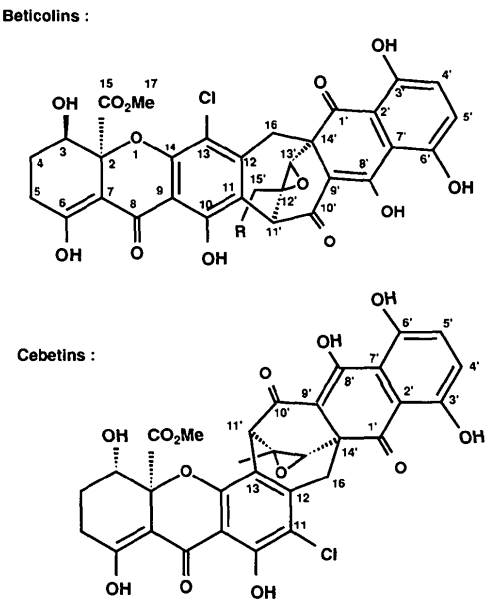
(*Received* 4 *March* 1994; *accepted* 26 *May* 1994)

Abstract

Several toxins were isolated from a mycelial extract of a *Cercospora beticola* strain. Two were obtained as monocrystals. They are isomorphous and represent the leading xanthraquinones, a new class of natural toxins. Beticolin 2 [8*R*-(8α, 14aα, 14β, 17aα)]-methyl 16-chloro-2′,5′,6′,9,11,14-hexahydroxy-(19-methyl-8,17a-oxirano)-1,7,8,12,13,14a,17a-octahydro-9*H*,17*H*-naphtho[2′,3′:1,2]cyclohepta[5,6-*c*]xanthene-14a-acetate: $C_{31}H_{23}ClO_{13}$, $M_r = 638.97$, orthorhombic, $P2_12_12_1$, $a = 20.230$ (6), $b = 18.921$ (5), $c = 8.205$ (3) Å, $V = 3140.6$ Å^3, $Z = 4$, $D_x = 1.352$ g cm^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, $\mu = 15.46$ cm^{-1}, $F(000) = 1320$, $T = 293$ K, structure solved by direct methods, $R = 0.062$ for 2480 observed reflections. Beticolin 4 [8*R*-(8α, 14aα, 14β, 17aα)]-methyl 16-chloro-2′, 5′, 6′, 9, 11, 14-hexahydroxy-(19-hydroxymethyl-8,17a-oxirano)-1,7,8,12,13,14a-octahydro-9*H*,17*H*-naphtho[2′,3′:1,2]cyclohepta[5,6-*c*]xanthene-14a-acetate: $C_{31}H_{23}ClO_{14}$, $M_r = 654.97$, orthorhombic, $P2_12_12_1$, $a = 20.585$ (5), $b = 18.721$ (4), $c = 8.206$ (3) Å, $V = 3162.3$ Å^3, $Z = 4$, $D_x = 1.375$ g cm^{-3}, λ(Cu $K\alpha$) = 1.5418 Å, $\mu = 15.71$ cm^{-1}, $F(000) = 1352$, $T = 293$ K, structure solved by isomorphous replacement, $R = 0.09$ for 1124 observed reflections. Biogenetic pathways relating beticolins to other subclasses of fungal metabolites (cebetins and xanthoquinodins) are detailed.

Introduction

Cercospora Beticola is a parasitic fungus responsible for leaf spot disease on sugar beet. The pathogen agent produces, in addition to the deep red cercosporin (Kuyama & Tamura, 1957; Lousberg, Weiss, Salemink, Arnone, Merlini & Nasini, 1971), a yellow compound, originally named the 'gelben fraktion', then CBT (Schlösser, 1962; Schlösser, 1971; Assante, Locci, Camarda, Merlini & Nasini, 1977). The structure of this fraction remained unknown for many years until we isolated two major yellow compounds from a mycelial extract of a *C. beticola* strain and solved their structures by X-ray analysis. They were named beticolins (Milat, Prangé, Ducrot, Tabet, Einhorn, Blein & Lallemand, 1992). Another structure was obtained by X-ray analysis (Jalal, Hossain, Robeson & van der Helm, 1992) which presents similarities with beticolins, but was isolated as a dimer complex of magnesium and named cebetin B. These unusual compounds revealed a new chlorinated heterodimer xanthone/anthraquinone skeleton (Scheme 1). All these yellow toxins belong to the two families of beticolins and cebetins, due to the way in which the two anthraquinone and xanthone precursors are attached together. Beticolin 4 (R = OH) differs from beticolin 2 (R = H) by a single additional alcohol function. They were crystallized from $CHCl_3$/methanol or CH_2Cl_2/methanol mixtures (the two structures are isomorphous). Recently, a new subclass of xanthoquinodins, a



Scheme 1. The chemical structure of beticolins and cebetins. Beticolins 2 (R = H) and 4 (R = OH) show the numbering adopted in this study.

* Author to whom correspondence should be addressed.

group of anticoccidal antibiotics, was discovered (Tabata, Tomoda, Matsuzaki & Omura, 1993) and their biosynthesis discussed and detailed by NMR. All these fungal metabolites have important biochemical properties; the xanthoquinodins are antibiotics, the beticolins and cebetins are toxic metabolites inhibiting a number of biochemical processes (Blein, Bourdil, Rossignol & Scalla, 1988; Milat, Fraichard, Blein & Pugin, 1992) and this explains why these ubiquitous biochemicals are still under intense evaluation.

Experimental

Isolation and purification of beticolins from *Cercospora beticola* have been previously reported (Milat, Blein, Einhorn, Tabet, Ducrot & Lallemand, 1993). About ten products were obtained from the crude mycelial extracts by careful chromatography in different phase/solvent systems (Milat, personal communication). Among these, one major yellow component (beticolin 2) and a minor toxin (beticolin 4) were crystallized in a suitable form for X-ray diffraction studies. Beticolins 2 and 4 were recrystallized as single crystals by slow evaporation of a (3:1) CH_2Cl_2/methanol solution at room temperature. In the case of beticolin 4, only very small crystals were obtained. The maximum length is *ca* 0.2 mm, but the thickness is never greater than 0.05 mm. The experimental details are reported in Table 1.

Data collections and refinements

Beticolin 2. Each reflection was scanned over a 1.2° angle width at a speed of 0.03° s^{-1}. The background was deduced from two stationary measurements on both sides of the reflection. The structure was solved by direct methods using *SHELXS*86 (Sheldrick, 1985). All the H atoms but one were located on difference-Fourier syntheses.

Beticolin 4. Reflections were measured using the step-scan technique (20 steps of 5 s each) with a speed reduced to 0.01° s^{-1}. The intensities were deduced from standard profile fitting analyses. The structure was solved by Fourier difference using the previously solved beticolin 2 structure. On the difference-Fourier map, the additional O atom at C(15′) was clearly observed. Owing to the small amount of available diffraction data, the structure was refined with only isotropic thermal factors, except for the Cl atom. H atoms were given theoretical positions when bonded to C atoms (13 over a total of 23).

The final positional parameters* are given in Table 2 for non-H atoms. The bond lengths and angles with estimated standard deviations are given in Tables 3 and 4. The numbering of the atomic positions is given in Scheme 1. Prime labelled atoms correspond to the anthraquinone precursor. Some of these labels are also reported in the biosynthetic pathway described in scheme 2 for clarity.

The *ORTEP* drawing (Johnson, 1976) of beticolin 2 is shown in Fig. 1 and Fig. 2 represents the *PLUTO* drawing (Motherwell & Clegg, 1978) of the beticolin 4 structure.

Table 1. *Experimental details*

Crystal data	Beticolin 2	Beticolin 4
Chemical formula	$C_{31}H_{23}ClO_{13}$	$C_{31}H_{23}ClO_{14}$
Molecular weight	638.97	654.97
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	20.230 (6)	20.585 (5)
b (Å)	18.921 (5)	18.721 (4)
c (Å)	8.205 (3)	8.206 (3)
Z	4	4
V (Å^3)	3140.6	3162.3
D_x (Mg m^{-3})	1.352	1.375
Absorption coefficient (cm^{-1})	15.46	15.71
$F(000)$	1320	1352
No. of reflections for lattice parameters	25	23
θ range for lattice parameters (°)	4–20	4–17
Radiation (Å)	1.5418 (Cu $K\alpha$)	1.5418 (Cu $K\alpha$)
Temperature (K)	290	295
Crystal size (mm)	0.4 × 0.2 × 0.2	0.2 × 0.05 × 0.05
Crystal colour	Yellow	Yellow
Crystal description	Hexagonal rods	Needles
Data collection		
Diffractometer	Philips PW1100	Philips PW1100
Collection method	$\theta/2\theta$ integrate scans	$\theta/2\theta$ step scans
Absorption correction type	Empirical	None
Absorption correction (T_{min}, T_{max})	0.7–1.6	—
No. of reflections measured	3233	3211
No. of independent reflections	2720	2510
No. of observed reflections	2480	1124
Criterion for observed	$I \geq 2\sigma(I)$	$I \geq 2\sigma(I)$
R_{int}	0.044	0.076
θ_{max} (°)	63	61
No. of standard reflections (and intervals)	3–2 h	3–2 h
Variation of standards (%)	≤ 4	15
h_{min}–h_{max}	0–21	0–20
k_{min}–k_{max}	0–20	0–18
l_{min}–l_{max}	0–10	0–9
Refinements		
Treatment of H atoms	Located – not refined	Theoretical – not refined
F; F^2 or I	F	F
R	0.062	0.091
wR	0.064	0.095
No. of parameters refined	491	253
No. of reflections used in refinement	2480	1124
Weighting scheme, w	$[\sigma^2(F) + 0.0005\sigma F^2]^{-1}$	$[\sigma^2(F) + 0.0004\sigma F^2]^{-1}$
$(\Delta/\sigma)_{max}$	0.09	0.08
$(\Delta\rho)_{min}$ (e Å^{-3})	−0.23	−0.26
$(\Delta\rho)_{max}$ (e Å^{-3})	0.18	0.24
Extinction correction method	None	None
Source of atomic X-ray factors	*International Tables for X-ray Crystallography* (1974, Vol. IV)	

* Lists of structure factors, anisotropic thermal parameters, coordinates of H atoms, and bond distances and angles have been deposited with the IUCr (Reference: PA0299). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Positional parameters* ($\times 10^4$) *and mean recalculated isotropic factors* ($\times 10^4$) *for non* H-*atoms*† *for beticolins* 2 *and* 4

	x	y	z	$\langle U \rangle$
Beticolin 2				
O(1)	−70 (2)	4671 (3)	998 (7)	27 (4)
C(2)	−788 (3)	4611 (4)	960 (10)	26 (6)
C(3)	−1044 (3)	5322 (4)	1572 (11)	28 (7)
O(3)	−774 (3)	5478 (3)	3134 (8)	40 (5)
C(4)	−1802 (4)	5284 (5)	1777 (13)	39 (8)
C(5)	−2002 (4)	4721 (5)	2991 (13)	41 (9)
C(6)	−1608 (3)	4067 (4)	2880 (10)	28 (6)
O(6)	−1843 (3)	3525 (3)	3716 (7)	35 (5)
C(7)	−1027 (3)	4006 (4)	2025 (9)	22 (6)
C(8)	−638 (4)	3373 (4)	2147 (10)	31 (7)
O(8)	−851 (3)	2828 (3)	2804 (8)	44 (6)
C(9)	47 (3)	3413 (4)	1550 (11)	30 (7)
C(10)	469 (3)	2831 (4)	1630 (10)	28 (6)
O(10)	263 (3)	2207 (3)	2155 (9)	44 (6)
C(11)	1131 (3)	2885 (4)	1128 (10)	30 (7)
C(12)	1381 (3)	3540 (4)	620 (9)	25 (6)
C(13)	959 (3)	4124 (4)	572 (10)	31 (7)
C(14)	297 (3)	4073 (4)	1026 (9)	20 (6)
C(15)	−966 (4)	4512 (4)	−877 (10)	32 (7)
O(15)	−1175 (5)	4980 (4)	−1683 (9)	86 (9)
C(16)	2086 (3)	3682 (4)	33 (11)	29 (7)
O(16)	−860 (5)	3883 (4)	−1356 (9)	80 (10)
C(17)	−1026 (10)	3719 (7)	−3059 (13)	125 (20)
C(1′)	3245 (4)	3414 (4)	−337 (9)	27 (7)
O(1′)	3301 (3)	3551 (3)	−1819 (7)	44 (6)
C(2′)	3772 (3)	3580 (4)	791 (9)	26 (6)
C(3′)	4329 (4)	3961 (4)	264 (10)	32 (7)
O(3′)	4400 (3)	4169 (4)	−1291 (7)	42 (6)
C(4′)	4824 (4)	4129 (4)	1383 (11)	34 (7)
C(5′)	4779 (4)	3939 (5)	2951 (11)	42 (8)
C(6′)	4239 (4)	3543 (4)	3516 (11)	36 (7)
O(6′)	4240 (3)	3385 (3)	5135 (8)	49 (6)
C(7′)	3724 (3)	3383 (4)	2482 (9)	27 (6)
C(8′)	3145 (4)	2976 (4)	3029 (9)	28 (6)
O(8′)	3123 (3)	2774 (3)	4517 (7)	41 (5)
C(9′)	2628 (3)	2820 (4)	1972 (9)	26 (6)
C(10′)	2112 (4)	2386 (4)	2469 (10)	30 (7)
O(10′)	2061 (3)	2127 (3)	3918 (7)	39 (5)
C(11′)	1573 (4)	2225 (4)	1290 (11)	30 (7)
C(12′)	1855 (4)	2046 (4)	−397 (12)	39 (8)
C(13′)	2399 (4)	2475 (4)	−913 (10)	31 (7)
O(13′)	2532 (3)	1763 (3)	−341 (8)	47 (6)
C(14′)	2604 (3)	3074 (4)	224 (10)	28 (6)
C(15′)	1405 (5)	1670 (6)	−1571 (13)	53 (10)
Cl(13)	1246 (1)	4948 (1)	5 (4)	52 (2)
Beticolin 4				
O(1)	−23 (8)	4731 (6)	1067 (9)	27 (5)
C(2)	−701 (8)	4665 (9)	863 (11)	33 (10)
C(3)	−987 (8)	5307 (7)	1328 (10)	27 (7)
O(3)	−780 (7)	5540 (6)	2904 (11)	26 (5)
C(4)	−1754 (8)	5287 (8)	1391 (11)	26 (8)
C(5)	−1965 (9)	4705 (8)	2660 (11)	31 (9)
C(6)	−1582 (8)	4094 (8)	2787 (11)	30 (9)
O(6)	−1796 (6)	3534 (6)	3572 (13)	22 (4)
C(7)	−960 (9)	3936 (7)	2004 (10)	28 (8)
C(8)	−548 (8)	3405 (8)	2170 (11)	24 (9)
O(8)	−804 (6)	2850 (6)	2899 (13)	46 (7)
C(9)	130 (8)	3404 (8)	1659 (9)	28 (8)
C(10)	512 (7)	2818 (7)	1658 (11)	28 (7)
C(11)	1139 (7)	2878 (7)	1241 (10)	21 (8)
C(12)	1403 (7)	3494 (7)	721 (12)	23 (7)
C(13)	971 (7)	4168 (7)	697 (12)	27 (7)
C(14)	331 (7)	4038 (6)	1109 (11)	22 (6)
C(15)	−819 (11)	4376 (10)	−884 (11)	50 (11)
C(16)	2123 (9)	3695 (9)	178 (10)	34 (9)
C(17)	−1392 (11)	3867 (11)	−3068 (12)	99 (16)
O(15)	−435 (11)	4391 (10)	−1815 (11)	153 (25)
O(16)	−1375 (11)	4266 (10)	−1425 (11)	152 (20)
O(10)	302 (7)	2199 (6)	2259 (13)	28 (5)
C(1′)	3229 (7)	3412 (7)	−323 (10)	25 (7)
O(1′)	3271 (7)	3595 (6)	−1838 (13)	33 (6)
C(2′)	3749 (7)	3604 (6)	787 (13)	25 (6)
C(3′)	4271 (8)	3952 (7)	362 (11)	28 (8)
O(3′)	4363 (7)	4181 (6)	−1392 (13)	31 (6)
C(4′)	4789 (9)	4172 (9)	1275 (11)	34 (10)
C(5′)	4764 (8)	3899 (7)	2714 (12)	26 (8)
C(6′)	4271 (9)	3531 (9)	3466 (10)	37 (10)
O(6′)	4286 (7)	3350 (6)	5069 (10)	54 (7)
C(7′)	3780 (9)	3355 (8)	2390 (11)	29 (9)
C(8′)	3176 (7)	2979 (7)	3035 (10)	25 (7)
O(8′)	3174 (7)	2713 (6)	4547 (10)	33 (6)
C(9′)	2648 (7)	2825 (7)	1940 (9)	21 (6)
C(10′)	2137 (8)	2399 (7)	2433 (16)	29 (8)
O(10′)	2100 (7)	2066 (6)	4001 (18)	38 (6)
C(11′)	1579 (8)	2219 (8)	1309 (12)	29 (9)
C(12′)	1871 (7)	2045 (6)	−338 (15)	22 (6)
C(13′)	2413 (8)	2480 (7)	−838 (16)	28 (7)
O(13′)	2499 (7)	1734 (6)	−339 (14)	32 (6)
C(14′)	2602 (8)	3070 (7)	206 (13)	21 (8)
C(15′)	1407 (8)	1626 (9)	−1390 (13)	31 (9)
O(15′)	1448 (8)	903 (8)	−854 (13)	66 (11)
Cl(13)	1268 (3)	4977 (3)	65 (7)	42 (4)

† Given in Å^2 and calculated as $\langle U \rangle = (1/3)\sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j$.

Table 3. *Distances* (Å) *for non*-H *atoms* (*e.s.d.'s in parentheses*) *for beticolins* 2 *and* 4

Beticolin 2			
O(1)—C(2)	1.456 (6)	C(1′)—O(1′)	1.248 (8)
O(1)—C(14)	1.353 (7)	C(1′)—C(2′)	1.446 (8)
C(2)—C(3)	1.527 (10)	C(1′)—C(14′)	1.520 (9)
C(2)—C(7)	1.519 (9)	C(2′)—C(3′)	1.405 (9)
C(2)—C(15)	1.562 (10)	C(2′)—C(7′)	1.440 (9)
C(3)—O(3)	1.424 (9)	C(3′)—O(3′)	1.343 (9)
C(3)—C(4)	1.546 (9)	C(3′)—C(4′)	1.396 (10)
C(4)—C(5)	1.513 (12)	C(4′)—C(5′)	1.339 (12)
C(5)—C(6)	1.474 (11)	C(5′)—C(6′)	1.404 (10)
C(6)—O(6)	1.322 (10)	C(6′)—O(6′)	1.362 (10)
C(6)—C(7)	1.374 (9)	C(6′)—C(7′)	1.377 (9)
C(7)—C(8)	1.436 (10)	C(7′)—C(8′)	1.472 (9)
C(8)—O(8)	1.241 (9)	C(8′)—O(8′)	1.280 (9)
C(8)—C(9)	1.471 (8)	C(8′)—C(9′)	1.390 (9)
C(9)—C(10)	1.396 (8)	C(9′)—C(10′)	1.391 (8)
C(9)—C(14)	1.415 (9)	C(9′)—C(14′)	1.513 (9)
C(10)—O(10)	1.324 (8)	C(10′)—O(10′)	1.290 (9)
C(10)—C(11)	1.404 (8)	C(10′)—C(11′)	1.488 (10)
C(11)—C(12)	1.402 (8)	C(11′)—C(12′)	1.534 (11)
C(11)—C(11′)	1.543 (9)	C(12′)—O(13′)	1.471 (9)
C(12)—C(13)	1.397 (8)	C(12′)—C(13′)	1.430 (9)
C(12)—C(16)	1.530 (8)	C(12′)—C(15′)	1.504 (12)
C(13)—C(14)	1.394 (8)	O(13′)—C(13′)	1.451 (9)
C(13)—Cl(13)	1.726 (8)	C(13′)—C(14′)	1.526 (9)
C(15)—O(15)	1.184 (9)	C(14′)—C(16)	1.565 (9)
C(15)—O(16)	1.271 (10)		
C(17)—O(16)	1.470 (12)		
Beticolin 4			
O(1)—C(2)	1.411 (21)	C(1′)—O(1′)	1.293 (12)
O(1)—C(14)	1.489 (17)	C(1′)—C(2′)	1.451 (17)
C(2)—C(3)	1.392 (19)	C(1′)—C(14′)	1.504 (19)
C(2)—C(7)	1.739 (18)	C(2′)—C(3′)	1.304 (19)
C(2)—C(15)	1.551 (16)	C(2′)—C(7′)	1.396 (14)
C(3)—C(4)	1.579 (19)	C(3′)—O(3′)	1.514 (14)
C(3)—O(3)	1.429 (14)	C(3′)—C(4′)	1.366 (18)
C(4)—C(5)	1.569 (16)	C(4′)—C(5′)	1.287 (14)
C(5)—C(6)	1.394 (20)	C(5′)—C(6′)	1.373 (19)
C(6)—C(7)	1.462 (20)	C(6′)—O(6′)	1.358 (10)
C(6)—O(6)	1.306 (15)	C(6′)—C(7′)	1.382 (19)
C(7)—C(8)	1.314 (19)	C(7′)—C(8′)	1.523 (19)
C(8)—C(9)	1.457 (20)	C(8′)—O(8′)	1.336 (11)
C(8)—O(8)	1.308 (16)	C(8′)—C(9′)	1.440 (16)
C(9)—C(10)	1.350 (16)	C(9′)—C(10′)	1.380 (19)
C(9)—C(14)	1.335 (18)	C(9′)—C(14′)	1.498 (12)
C(10)—C(11)	1.340 (20)	C(10′)—O(10′)	1.432 (18)

Table 3 (*cont.*)

C(10)—O(10)	1.332 (16)	C(10′)—C(11′)	1.512 (19)
C(11)—C(12)	1.344 (18)	C(11′)—C(12′)	1.514 (16)
C(11)—C(11′)	1.531 (20)	C(12′)—C(13′)	1.440 (19)
C(12)—C(13)	1.545 (19)	C(12′)—O(13′)	1.417 (19)
C(12)—C(16)	1.592 (20)	C(12′)—C(15′)	1.508 (18)
C(13)—C(14)	1.380 (18)	C(13′)—O(13′)	1.466 (16)
C(13)—Cl(13)	1.713 (12)	C(13′)—C(14′)	1.451 (16)
C(15)—O(15)	1.099 (20)	C(15′)—O(15′)	1.426 (20)
C(15)—O(16)	1.244 (26)	C(14′)—C(16′)	1.530 (20)
C(17)—O(16)	1.542 (18)		

Table 4. *Bond angles* (°) *for non*-H *atoms* (*e.s.d.'s given in parentheses*) *for beticolins* 2 *and* 4

Beticolin 2

C(2)—O(1)—C(14)	118.8 (7)	C(2′)—C(1′)—C(14′)	121.8 (8)
O(1)—C(2)—C(3)	105.2 (9)	C(1′)—C(2′)—C(3′)	120.3 (9)
O(1)—C(2)—C(7)	111.4 (9)	C(1′)—C(2′)—C(7′)	120.7 (8)
O(1)—C(2)—C(15)	105.1 (10)	C(3′)—C(2′)—C(7′)	118.9 (10)
C(3)—C(2)—C(7)	111.6 (10)	C(2′)—C(3′)—O(3′)	121.9 (10)
C(3)—C(2)—C(15)	110.2 (10)	C(2′)—C(3′)—C(4′)	119.3 (10)
C(7)—C(2)—C(15)	113.0 (9)	O(3′)—C(3′)—C(4′)	118.8 (10)
C(2)—C(3)—O(3)	110.3 (9)	C(3′)—C(4′)—C(5′)	121.5 (10)
C(2)—C(3)—C(4)	109.3 (9)	C(4′)—C(5′)—C(6′)	120.9 (11)
O(3)—C(3)—C(4)	107.0 (8)	C(5′)—C(6′)—O(6′)	116.0 (11)
C(3)—C(4)—C(5)	111.7 (11)	C(5′)—C(6′)—C(7′)	120.2 (10)
C(4)—C(5)—C(6)	114.0 (11)	O(6′)—C(6′)—C(7′)	123.6 (10)
C(5)—C(6)—O(6)	115.1 (10)	C(2′)—C(7′)—C(6′)	119.0 (10)
C(5)—C(6)—C(7)	124.4 (10)	C(2′)—C(7′)—C(8′)	118.9 (9)
O(6)—C(6)—C(7)	120.5 (10)	C(6′)—C(7′)—C(8′)	121.9 (10)
C(2)—C(7)—C(6)	120.2 (9)	C(7′)—C(8′)—O(8′)	118.3 (9)
C(2)—C(7)—C(8)	119.6 (9)	C(7′)—C(8′)—C(9′)	121.3 (9)
C(6)—C(7)—C(8)	120.2 (10)	O(8′)—C(8′)—C(9′)	120.4 (10)
C(7)—C(8)—O(8)	122.2 (11)	C(8′)—C(9′)—C(10′)	120.5 (10)
C(7)—C(8)—C(9)	116.8 (11)	C(8′)—C(9′)—C(14′)	123.2 (9)
O(8)—C(8)—C(9)	120.9 (11)	C(10′)—C(9′)—C(14′)	116.2 (8)
C(8)—C(9)—C(10)	121.4 (11)	C(9′)—C(10′)—O(10′)	123.7 (10)
C(8)—C(9)—C(14)	118.9 (9)	C(9′)—C(10′)—C(11′)	118.7 (10)
C(10)—C(9)—C(14)	119.5 (10)	O(10′)—C(10′)—C(11′)	117.6 (10)
C(9)—C(10)—O(10)	121.7 (10)	C(11)—C(11′)—C(10′)	108.4 (9)
C(9)—C(10)—C(11)	120.8 (10)	C(11)—C(11′)—C(12′)	108.4 (9)
O(10)—C(10)—C(11)	117.5 (10)	C(10′)—C(11′)—C(12′)	111.1 (8)
C(10)—C(11)—C(12)	119.7 (10)	C(11′)—C(12′)—O(13′)	113.4 (10)
C(10)—C(11)—C(11′)	117.9 (11)	C(11′)—C(12′)—C(13′)	115.3 (10)
C(12)—C(11)—C(11′)	122.2 (10)	C(11′)—C(12′)—C(15′)	117.2 (10)
C(11)—C(12)—C(13)	119.2 (10)	O(13′)—C(12′)—C(13′)	60.0 (9)
C(11)—C(12)—C(16)	125.8 (11)	O(13′)—C(12′)—C(15′)	114.3 (10)
C(13)—C(12)—C(16)	115.0 (9)	C(13′)—C(12′)—C(15′)	122.9 (12)
C(12)—C(13)—C(14)	121.7 (10)	C(12′)—O(13′)—C(13′)	58.6 (7)
C(12)—C(13)—Cl(13)	121.1 (9)	C(12′)—C(13′)—O(13′)	61.4 (7)
C(14)—C(13)—Cl(13)	117.2 (9)	C(12′)—C(13′)—C(14′)	116.7 (10)
O(1)—C(14)—C(9)	123.2 (9)	O(13′)—C(13′)—C(14′)	116.2 (9)
O(1)—C(14)—C(13)	117.7 (9)	C(1′)—C(14′)—C(9′)	113.2 (9)
C(9)—C(14)—C(13)	119.1 (8)	C(1′)—C(14′)—C(13′)	111.2 (8)
C(2)—C(15)—O(15)	122.1 (12)	C(1′)—C(14′)—C(16)	103.3 (8)
C(2)—C(15)—O(16)	111.8 (10)	C(9′)—C(14′)—C(13′)	110.6 (8)
O(15)—C(15)—O(16)	126.0 (14)	C(9′)—C(14′)—C(16)	110.5 (8)
C(15)—O(16)—C(17)	116.9 (14)	C(13′)—C(14′)—C(16)	107.6 (9)
O(1′)—C(1′)—C(2′)	120.8 (10)	C(12′)—C(16)—C(14′)	117.6 (9)
O(1′)—C(1′)—C(14′)	117.4 (10)		

Beticolin 4

C(2)—O(1)—C(14)	114.2 (14)	C(12′)—C(15′)—O(15′)	106.2 (16)
O(1)—C(2)—C(3)	108.1 (14)	C(9′)—C(14′)—C(13′)	110.1 (12)
O(1)—C(2)—C(7)	107.9 (12)	C(1′)—C(14′)—C(13′)	112.6 (12)
O(1)—C(2)—C(15)	107.2 (14)	C(1′)—C(14′)—C(9′)	110.5 (12)
C(3)—C(2)—C(7)	113.6 (12)	C(16)—C(14′)—C(13′)	113.6 (14)
C(3)—C(2)—C(15)	119.2 (16)	C(16)—C(14′)—C(9′)	106.8 (14)
C(7)—C(2)—C(15)	100.1 (14)	C(16)—C(14′)—C(1′)	102.9 (14)
C(2)—C(3)—C(4)	114.3 (12)	C(12′)—O(13′)—C(13′)	59.9 (12)
C(2)—C(3)—O(3)	112.7 (14)	O(13′)—C(13′)—C(14′)	121.9 (12)
C(4)—C(3)—O(3)	106.0 (14)	C(12′)—C(13′)—C(14′)	118.0 (12)
C(3)—C(4)—C(5)	108.3 (14)	C(12′)—C(13′)—O(13′)	58.4 (12)
C(4)—C(5)—C(6)	117.6 (16)	O(13′)—C(12′)—C(15′)	111.3 (13)
C(5)—C(6)—C(7)	128.9 (16)	C(13′)—C(12′)—C(15′)	128.4 (15)

Table 4 (*cont.*)

C(5)—C(6)—O(6)	120.3 (16)	C(13′)—C(12′)—O(13′)	61.7 (13)
C(7)—C(6)—O(6)	110.4 (16)	C(11′)—C(12′)—C(15′)	111.8 (16)
C(2)—C(7)—C(6)	110.2 (14)	C(11′)—C(12′)—O(13′)	116.8 (16)
C(2)—C(7)—C(8)	116.9 (14)	C(11′)—C(12′)—C(13′)	116.2 (14)
C(6)—C(7)—C(8)	132.3 (16)	C(10′)—C(11′)—C(12′)	106.9 (14)
C(7)—C(8)—C(9)	126.1 (16)	C(11)—C(11′)—C(12′)	112.0 (16)
C(7)—C(8)—O(8)	112.9 (16)	C(11)—C(11′)—C(10′)	106.9 (14)
C(9)—C(8)—O(8)	121.1 (14)	O(10′)—C(10′)—C(11′)	114.2 (14)
C(8)—C(9)—C(10)	123.9 (14)	C(9′)—C(10′)—C(11′)	121.9 (14)
C(8)—C(9)—C(14)	113.2 (15)	C(9′)—C(10′)—O(10′)	123.8 (14)
C(10)—C(9)—C(14)	122.8 (14)	C(10′)—C(9′)—C(14′)	114.1 (14)
C(9)—C(10)—C(11)	119.5 (14)	C(8′)—C(9′)—C(14′)	125.4 (16)
C(9)—C(10)—O(10)	121.2 (16)	C(8′)—C(9′)—C(10′)	120.5 (14)
C(11)—C(10)—O(10)	118.7 (17)	O(8′)—C(8′)—C(9′)	120.1 (14)
C(10)—C(11)—C(12)	122.8 (16)	C(7′)—C(8′)—C(9′)	119.4 (14)
C(10)—C(11)—C(11′)	119.5 (17)	C(7′)—C(8′)—O(8′)	119.9 (14)
C(12)—C(11)—C(11′)	117.7 (15)	C(6′)—C(7′)—C(8′)	119.0 (16)
C(11)—C(12)—C(13)	118.3 (15)	C(2′)—C(7′)—C(8′)	116.4 (14)
C(11)—C(12)—C(16)	131.9 (14)	C(2′)—C(7′)—C(6′)	123.8 (12)
C(13)—C(12)—C(16)	109.8 (15)	O(6′)—C(6′)—C(7′)	125.2 (14)
C(12)—C(13)—C(14)	113.6 (15)	C(5′)—C(6′)—C(7′)	111.9 (16)
C(12)—C(13)—Cl(13)	121.4 (18)	C(5′)—C(6′)—O(6′)	122.9 (16)
C(14)—C(13)—Cl(13)	124.8 (16)	C(4′)—C(5′)—C(6′)	129.8 (18)
O(1)—C(14)—C(9)	129.1 (16)	C(3′)—C(4′)—C(5′)	110.6 (12)
O(1)—C(14)—C(13)	107.8 (14)	O(3′)—C(3′)—C(4′)	109.8 (12)
C(9)—C(14)—C(13)	122.4 (14)	C(2′)—C(3′)—C(4′)	130.3 (13)
C(2)—C(15)—O(15)	121.3 (17)	C(2′)—C(3′)—O(3′)	119.9 (14)
C(2)—C(15)—O(16)	122.1 (13)	C(3′)—C(2′)—C(7′)	112.4 (14)
O(15)—C(15)—O(16)	114.7 (15)	C(1′)—C(2′)—C(7′)	122.9 (16)
C(12)—C(16)—C(14′)	114.5 (14)	C(1′)—C(2′)—C(3′)	124.3 (12)
C(15)—O(16)—C(17)	114.4 (15)	C(2′)—C(1′)—C(14′)	123.9 (14)
O(1′)—C(1′)—C(2′)	119.2 (14)	O(1′)—C(1′)—C(14′)	116.6 (14)

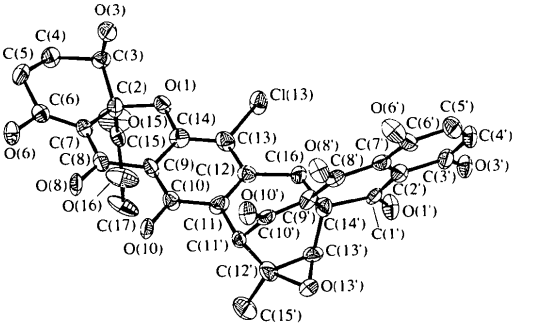


Fig. 1. *ORTEP* (Johnson, 1976) view of beticolin 2. The thermal ellipsoids are drawn at the 50% probability level.

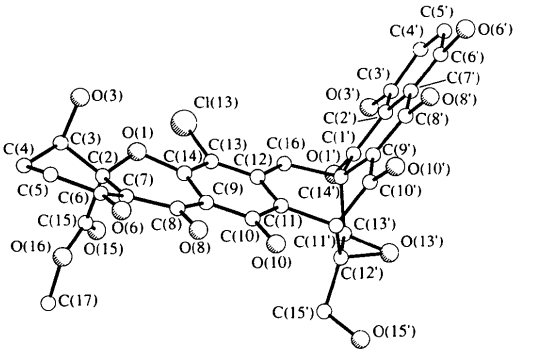
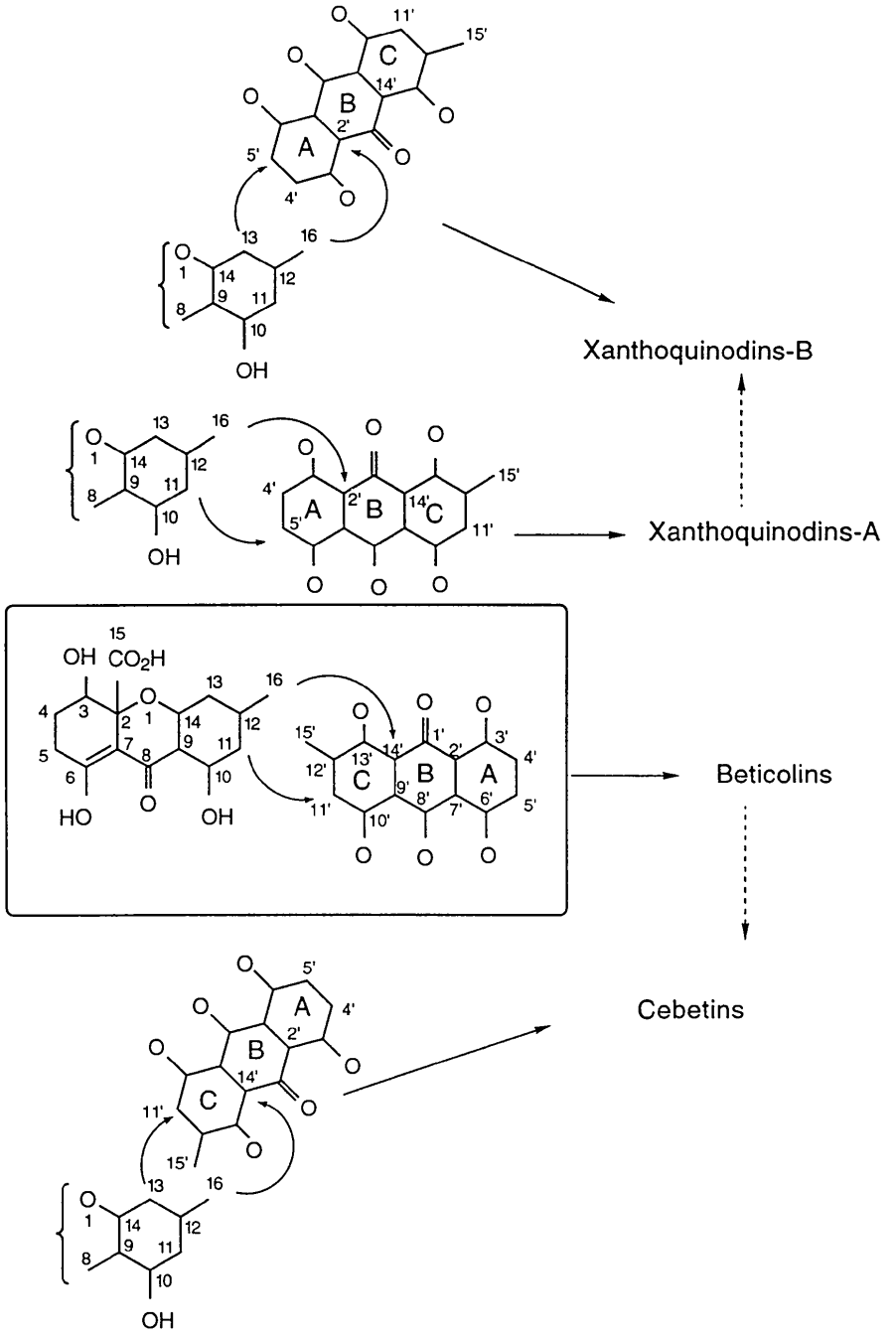


Fig. 2. *PLUTO* (Motherwell & Clegg, 1978) ball-and-stick view of the beticolin 4 structure. As indicated in the tables, only the Cl and O atoms were anisotropically refined, due to the paucity of the data. These atoms are represented as shadowed standard spheres.



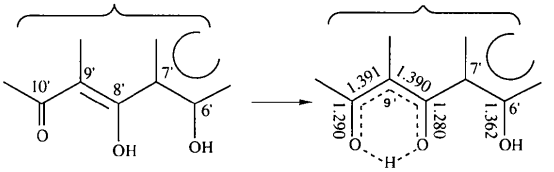
Scheme 2. Keto–enol tautomerism in the xanthone subunit of beticolins 2 and 4 as deduced from distances. The reported values are from the beticolin 2 structure.

The absolute configuration of beticolin 2, using the anomalous contribution of the Cl atom was tentatively investigated after refining the two enantiomers. A selected subset of 100 sorted Bijvoet pairs [$I \geq 3\sigma(I)$] according to the highest value for $Fc_{\mathbf{H}} - Fc_{\bar{\mathbf{H}}}$, gave $R = 0.059$ ($wR = 0.061$) and $R = 0.061$ ($wR = 0.063$), respectively. This is quite close to ascertain the absolute configuration without ambiguity, but the best result corresponds to the configuration determined for cebetin (Jalal *et al.*,1992) with the aid of the complexed Mg cations. This configuration is given in Scheme 1.

Discussion

The X-ray analysis revealed that beticolin 2 was an unusual polycyclic chlorinated compound with a very high degree of aromaticity and a rather unusual epoxide bridge (Scheme 1 and Fig. 1). The overall molecule

displays a V-shaped conformation due to the seven-membered ring bridged by the epoxide situated in the middle of the aromatic systems. Prior to this X-ray analysis, little information concerning these toxins was available. It was known from spectroscopic experiments that several phenol functions were present. Fig. 1 reveals that five phenol/quinone motifs are attached to the molecule and only one alcohol function at C(3), with a different stereochemistry in beticolins and cebetins, with respect to the angular carbomethoxy group at C(2). As several quinone groups are present in the vicinity of phenols, only careful examination of the C—O bond distances, as well as the location of the phenolic H atoms, gave the correct attribution for the quinone/phenol functions. As observed in many flavonoids (Liebich, 1979; Neuman, Becquart, Gillier, Leroux, Queval & Moretti, 1989), most of these phenolic H atoms are implicated in strong intramolecular hydrogen bonds toward the neighbouring keto group. In one case, the O(8′) and O(10′) atoms (Scheme 3), it was not possible to distinguish the carbonyl group from the phenol group due to electronic resonance, thus giving a stable tautomeric form. In cebetins, the two sites of magnesium complexation involve, in addition to this particular group, the OH(6)–CO(8) motif of the xanthone subunit. It is interesting to note that in cebetins, only the second complexation site is a resonant tautomer.



Scheme 3. The biogenetic pathway of the dimerization of the xanthone and anthraquinone monomers in all the known subclasses of xanthraquinones metabolites: beticolins, cebetins, xanthoquinodins *A* and *B*.

The packing of beticolin 2 is shown in Fig. 3. Despite the high number of possible hydrogen bonds, there are only a few weak polar interactions in the crystal packing, which is dominated mainly by the staggering interactions of the aromatic rings. The C(15′) methyl group, which bears the additional hydroxyl group in beticolin 4, is situated in a hydrophobic pocket without interactions. This absence of contacts explains the isomorphous cell adopted by the hydroxylated beticolin 4.

This molecular structure, analysed together with the recently determined cebetin and xanthoquinodin structures, allows us to define a new class of heterodimers, the xanthraquinones, which all share a common biosynthesis. This biosynthetic pathway requires in the final stage, the dimerization of two subunits, a xanthone and an anthraquinone. These two moieties are biogentically linked through C(16)—C(14′) and C(11)—C(11′) bond formation (Scheme 2) either by *re–re* linkage giving entry to the beticolin family or *re–si* association through [C(16)—C(14′) and C(13)—C(11′)], leading to the cebetin group (Arnone, Nasini, Merlini, Ragg & Assante, 1993). In the subclass of xanthoquinodins (Tabata *et al.*, 1993), it is interesting to note that these two different types of dimerizations already exist, leading to the xanthoquinodins A and B families. The main difference arises from the anthraquinone moiety which is now attached upside-down at the C(2′) and (C5′) positions of xanthone. The C(15′) anthraquinone methyl group is no longer included in the epoxy-bridge biogenetically derived from O(13′), but located on the other side of the molecule. Independently, Tabata *et al.* (1993), Milat *et al.* (1993) and Ducrot, Blein, Milat & Lallemand (1994) demonstrated that the elements of the beticolin group can be chemically converted into the cebetin group. The dashed arrows in Scheme 3 represent the chemical transformations that are possible within the subclasses, either by thermal or acid treatments. Time-dependent studies of *Cercospora beticola* toxin biosyntheses (Milat & Blein, 1994) revealed that several other related metabolites are synthesized prior to the final accumulation of the two major products beticolin 2 and cebetin *A* and it remains unclear if, biogenetically, beticolins can also be converted to cebetins as can be done by chemical means.

Concluding remarks

The X-ray structures of the *C. beticola* metabolites led to the discovery of a new class of toxins. It was apparent, from the cebetin structures, solved at the same time and from the more recently available xanthoquinodin structures, that all these metabolites share a common hetero dimerization process, using all the four possible orientations for the anthraquinone subunit. The beticolin/cebetin subclasses possess a very uncommon Cl atom

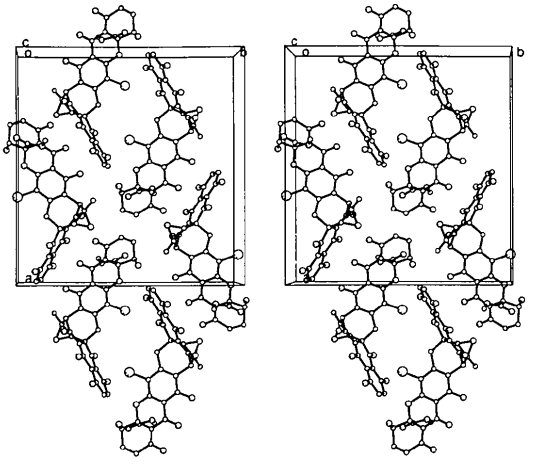


Fig. 3. Stereoview of the beticolin 2 packing (*PLUTO*, Motherwell & Clegg, 1978).

and show completely different properties for cation complexation and polar interactions (Mikes, Milat, Pugin & Blein, 1994; Mikes, Lavernet, Milat, Collange, Pâris & Blein, 1994).

We express our thanks to the Interface Chimie-Biologie du CNRS and Conseil Régional de Bourgogne for financial support, Professor J. C. Tabet and Drs J. Einhorn and C. Descoins for their interest and useful discussions.

References

ARNONE, A., NASINI, G., MERLINI, L., RAGG, E. & ASSANTE, G. (1993). *J. Chem. Soc. Perkin Trans* I, 145–151.
ASSANTE, G., LOCCI, R., CAMARDA, L., MERLINI, L. & NASINI, G. (1977). *Phytochemistry*, **16**, 243–247.
BLEIN, J. P., BOURDIL, I., ROSSIGNOL, M. & SCALLA, R. (1988). *Plant Physiol.* **88**, 429–434.
DUCROT, P. H., BLEIN, J. P., MILAT, M. L. & LALLEMAND, J. Y. (1994). *J. Chem. Soc. Chem. Commun.* pp. 2215–2216.
JALAL, M. A. F., HOSSAIN, M. B., ROBESON, D. J. & VAN HER HELM, D. (1992). *J. Am. Chem. Soc.* **114**, 5967–5971.
JOHNSON, C. K. (1976). *ORTEP*II. Report ONRL-5138. Oak Ridge National Laboratory, Tennessee, USA.
KUYAMA, S. & TAMURA, T. (1957). *J. Am. Chem. Soc.* **79**, 5726–5729.
LIEBICH, B. W. (1979). *Acta Cryst.* B**35**, 1186–1190.
LOUSBERG, R. J., WEISS, U., SALEMINK, C. A., ARNONE, A., MERLINI, L. & NASINI, G. (1971). *J. Chem. Soc. Chem Commun.* pp. 1463–1464.
MIKES, V., LAVERNET, S., MILAT, M. L., COLLANGE, E., PÂRIS, M. & BLEIN, J. P. (1994). *Biophys. Chem.* **52**, 259–265.
MIKES, V., MILAT, M. L., PUGIN, A. & BLEIN, J. P. (1994). *Biophys. Biochem. Acta*, **1195**, 124–130.
MILAT, M. L. & BLEIN, J. P. (1994). *Plant Physiol. Biochem.* In preparation.
MILAT, M. L., BLEIN, J. P., EINHORN, J., TABET, J. C., DUCROT, P. H. & LALLEMAND, J. Y. (1993). *Tetrahedron Lett.* **34**(9), 1483–1486.
MILAT, M. L., FRAICHARD, A., BLEIN, J. P. & PUGIN, A. (1992). Int. Workshop on Plant Membrane Biology, Monterey, July 19–24.
MILAT, M. L., PRANGÉ, T., DUCROT, P. H., TABET, J. C., EINHORN, J., BLEIN, J. P. & LALLEMAND, J. Y. (1992). *J. Am. Chem Soc.* **114**, 1478–1479.
MOTHERWELL, W. D. S. & CLEGG, W. (1978). *PLUTO. Program for Plotting Molecular and Crystal Structures*. Univ. of Cambridge, England.
NEUMAN, A., BECQUART, J., GILLIER, H., LEROUX, Y., QUEVAL, P. & MORETTI, J. L. (1989). *Acta Cryst.* C**45**, 1966–1970.
SCHLÖSSER, E. (1962). *Phytopathol. Z.* pp. 295–312.
SCHLÖSSER, E. (1971). *Phytopathol. Mediterr.* **10**, 154–158.
SHELDRICK, G. M. (1985). *SHELXS*86. *Program for the Solution of Crystal Structures*. Univ. of Göttingen, Germany.
TABATA, N., TOMODA, H., MATSUZAKI, K. & OMURA, S. (1993). *J. Am. Chem. Soc.* **115**, 8558–8564.

Acta Cryst. (1995). B**51**, 314–328

Use of the Estimated Errors of the Data in Structure-Correlation Studies

BY OLIVIERO CARUGO

Dipartimento di Chimica Generale, Università di Pavia, Italy

(*Received* 9 *June* 1994; *accepted* 3 *October* 1994)

Abstract

Novel statistical and numerical methods of data analysis, which make extensive use of the estimated errors (e.s.d.'s) of the data are presented and applied to structure-correlation problems. The novel procedures concern both univariate (histogram representation, HR) and multivariate (cluster analysis, CA, and principal-component analysis, PCA) statistical techniques. In the case of HR, the problem of optimally selecting the dimensions of the spaces is bypassed by convoluting a series of normal functions. In the case of CA, a probability significance is given to the similarity between two (or more than two) objects. In the case of PCA, a cross-validation technique, which takes into account the e.s.d.'s of the row data, allows the determination of the dimensionality of the principal-component space, easy detection of outliers with respect to any principal component, and evaluation of a more comprehensive percentage of the variance described by the principal components.

1. Introduction

An impressive growth of interest in structure-correlation studies appeared in the last two decades (Bürgi & Dunitz, 1994; Auf der Heyde, 1994; Orpen, 1993; Bürgi, 1992; Ferretti, Dubler-Steudle & Bürgi, 1992; Domenicano, 1992). This can be considered a consequence of two main factors: on one hand, structural determinations became very fast, and consequently the number of new structures increased; on the other hand, structural data have been organized in computer-readable databases (Allen, Bergerhoff & Sievers, 1987).

Many papers have been published on data-treatment strategies (Taylor & Allen, 1994) and, in particular, certain interest has been focused on the importance and use of estimated standard deviations (e.s.d.'s) of the structural data [bond distances and angles, torsions, *etc.* (Taylor & Kennard, 1983, 1985, 1986; Mackenzie, 1974; Hamilton & Abrahams, 1970, 1972; Abrahams & Keve, 1971; Abrahams, Hamilton & Mathieson, 1970)]. The importance of the e.s.d.'s is, in fact, crucial for