Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Thiochrome: A Convenient Synthesis from Thiamine[1]

Although the synthesis of thiochrome is readily attained by either the oxidation of thiamine by alkaline potassium ferricyanide[2] or the thermal decomposition of thiamine disulfide[3], its isolation from these procedures is inconvenient.

Studies by METZLER and MAIER[4] on the reactions of base with thiamine have disclosed that a tricyclic form of thiamine can be isolated in absolute alcohol. This study and also the observation by WOSTMANN and KNIGHT[5] that potassium ferricyanide in the presence of methyl alcohol favors a higher conversion of thiamine to thiochrome led us to attempt a simple oxidation of the vitamin in absolute methanol. The best oxidation procedure is as follows: Thiamine (1.68 g, 0.005 m) is suspended in absolute methyl alcohol (50 cm³). Iodine (1.27 g, 0.005 m) is then added and the mixture is stirred until all of the iodine has dissolved. At this point, potassium carbonate (4.14 g, 0.03 m) is added, and the mixture is stirred for 30 min. By this time the iodine color has discharged, and the bright yellow solution is then filtered to remove unreacted carbonate and potassium iodide. After filtration the solution is neutralized by the addition of acetic acid. On concentrating the filtrate at room temperature, crystals of thiochrome (0.265 g) (λ_{max} (EtOH) = 375; log ε = 4.20) are deposited. The melting point of the crude product ranges from 220–224°. The product was purified by recrystallization from chloroform. On further concentration of the filtrate, thiamine disulfide (0.138 g) (λ_{max} (EtOH) = 235, 275; log ε = 4.15, 3.80) was also isolated. The reaction time and neutralization step are critical. When the reaction time was lengthened to 1 h, it was found that the major products were thiazolone and disulfide. Similar results were also noted when the reaction mixture was concentrated without neutralizing the excess base. The oxidizing agents, nitrobenzene and nitrosobenzene, can also be substituted for iodine in the reaction. Under these conditions, the oxidizing agents are converted to azoxybenzene; thiamine is converted to thiochrome and disulfide.

Zusammenfassung. Die Arbeit gibt eine chemische Beschreibung des Präparates Thiochrom.

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Department of Biochemistry, Louisiana State University, Baton Rouge (Louisiana USA), January 11, 1965.

[1] This investigation was supported in part by Public Health Service Research Grant AM-08590-1 from the Arthritis and Metabolic Diseases Program.

[2] G. BARGER, F. BERGEL, and A. R. TODD, Nature *136*, 259 (1935); Ber. dtsch. chem. Ges. *68*, 2257 (1935). – R. KUHN and H. VETTER, Ber. dtsch. chem. Ges. *68*, 2375 (1935).

[3] O. ZIMA and R. R. WILLIAMS, Ber. dtsch. chem. Ges. *73*, 941 (1940). – P. SYKES and A. R. TODD, J. chem. Soc. *1951*, 534.

[4] G. D. MAIER and D. E. METZLER, J. Am. chem. Soc. *79*, 4386 (1957).

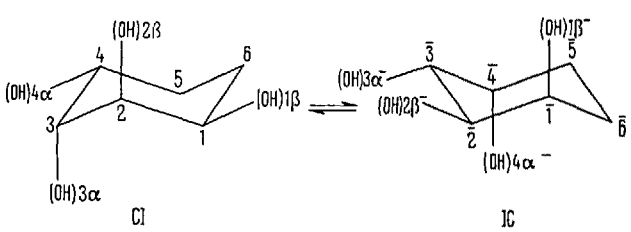
[5] B. S. WOSTMANN and P. T. KNIGHT, Exper. *16*, 500 (1960).

Molecular Rotations of Polyhydroxycyclohexanes in Relation to their Structures[1]

(–)-1,2/3,4-Cyclohexanetetrol

By applying the PM-method[2], the molecular rotation of (–)-1,2/3,4-cyclohexanetetrol is to be considered. The polyhydroxycyclohexanes and their observed molecular rotations are listed in Table I.

As is apparent in the Figure, (–)-1,2/3,4-cyclohexanetetrol has always two axial OH groups (which are *trans* to each other) and two equatorial OH groups (which are also *trans* to each other) in both the C 1- and 1 C-conformations. Therefore, the energy due to the interactions between four OH groups and the cyclohexane ring (including its H atoms) may be nearly equal in the two conformations. The number of intramolecular hydrogen bonds is, however, different. Concretely speaking, there are two adjacent *cis* hydrogen bonds in C 1 conformation (one is between $(OH)^{1\beta}$ and $(OH)^{2\beta}$ and the other is between $(OH)^{3\alpha}$ and $(OH)^{4\alpha}$). On the other hand, in 1 C conformation, there are two adjacent *cis* hydrogen bonds (one is between $(OH)^{1\beta-}$ and $(OH)^{2\beta-}$ and the other is between $(OH)^{3\alpha-}$ and $(OH)^{4\alpha-}$) and moreover there is one *trans* hydrogen bond (between $(OH)^{2\beta-}$ and $(OH)^{3\alpha-}$). Therefore it can be presumed that this *trans* hydrogen bond makes the 1 C conformation more stable than the C 1 conformation. In the equilibrium C 1 $\rightleftarrows$ 1 C, accordingly, the concentration of the 1 C conformation, [1 C],



C1 1C

[1] Part III. Part II, see S. YAMANA, Bull. chem. Soc. Japan *34*, 1212 (1961).

[2] S. YAMANA, J. Am. chem. Soc. *86*, 1606 (1964).

Table I

Cyclohexane	Conformation	Orientation of unit group (OH)	$[M]_D^{20}$(W)	Ref.
(−)-1/2-diol	C 1	[(1β), (2α)]	− 54.0°C	3
(−)-1,2/3,4-tetrol	C 1	[(1β), (2β), (3α), (4α)]	− 109.2°C	4
	1 C	[(1β⁻), (2β⁻), (3α⁻), (4α⁻)][6]		
	C 1	[(2β), (3β), (4α), (5α)]		
(−)-1,2,3,5/4-pentol	C 1	[(1β), (2β), (3β), (4α), (5β)]	− 8.9°C (at 27°C)	5
	1 C	[(1β⁻), (2β⁻), (3β⁻), (4α⁻), (5β⁻)][6]		
	C 1	[(2β), (3β), (4β), (5α), (6β)]		

may be larger than that of the C 1 conformation, [C 1], and the value of the conformational equilibrium constant, K (=[1 C]/[C 1]), may be greater than one. In order to estimate its approximate value, the PM-method is useful.

As is apparent in Table I, $\Sigma[\mu]_{D\,obs}^{20}$ of (−)-1,2/3,4-cyclohexanetetrol is given by the following two equations.

$$\Sigma[\mu]_{D\,obs}^{20} \text{ of C 1 conformation} = (1\beta)\lambda(2\beta) + (1\beta)\lambda(3\alpha) + (1\beta)\lambda(4\alpha) + (2\beta)\lambda(3\alpha) + (2\beta)\lambda(4\alpha) + (3\alpha)\lambda(4\alpha) \quad (1)$$

$$\Sigma[\mu]_{D\,obs}^{20} \text{ of 1 C conformation} = (2\beta)\lambda(3\beta) + (2\beta)\lambda(4\alpha) + (2\beta)\lambda(5\alpha) + (3\beta)\lambda(4\alpha) + (3\beta)\lambda(5\alpha) + (4\alpha)\lambda(5\alpha) \quad (2)$$

On the other hand, the values of $[\mu]_{D\,calcd}^{20}\{3/(n^2+2)\}$ caused by the dynamic coupling effect between any two members of unit groups in polyhydroxycyclohexane of C 1 conformation were already calculated by the fundamental formulae of the PM-method and given in Table II of the previous paper[7]. This is shown again here as Table II.

Owing to the following reasons, however, new values of $[\mu]_{D\,obs}^{20}$ are computed and shown in the square bracket.

In the previous paper[7], all of the polyhydroxycyclohexanes were assumed to exist entirely in C 1 conformation (i.e. the number of equatorial OH groups becomes as many as possible) and the value of ζ_{OH}^2 was then computed as $3.6402\{3/(n^2+2)\}$ by using the value of $[M]_D$(W) of (−)-1,3/2,4-cyclohexanetetrol. But, strictly speaking, (−)-1,3/2,4-cyclohexanetetrol is considered to exist in both of two conformations (C 1 and 1 C)[8] in its water solution, and accordingly the true value of ζ_{OH}^2 should be re-calculated by using another more appropriate substance.

Table II. $[\mu]_{D\,calcd}^{20}\{3/(n^2+2)\}$ and $[\mu]_{D\,obs}^{20}$ caused by the dynamic coupling effect between any two members of unit groups in polyhydroxycyclohexane of C 1 conformation

	6β	6α	5β	5α	4β	4α	3β	3α	2β	2α
1α	0	−A	−B	0	0	0	B	0	0	A
1β	−A	A	0	B	0	0	0	−B	A	−A
2α	−B	0	0	0	B	0	A	−A		
2β	0	B	0	0	0	−B	−A	0		
3α	0	0	B	0	0	A				
3β	0	0	0	−B	A	−A				
4α	B	0	A	−A						
4β	0	−B	−A	0						
5α	0	A								
5β	A	−A								

Note:	$[\mu]_{D\,calcd}^{20}\{3/(n^2+2)\}$	$[\mu]_{D\,obs}^{20}$
A	11.73	42.7 [55.5][9]
B	1.88	6.8 [8.9][9]

Referring to Tables I and II, $\Sigma[\mu]_{D\,obs}^{20}$ of (−)-1,2,3,5/4-cyclohexanepentol is given by the next equations.

$$\Sigma[\mu]_{D\,obs}^{20} \text{ of C 1 conformation} = (1\beta)\lambda(2\beta) + (1\beta)\lambda(3\beta) + (1\beta)\lambda(4\alpha) + (1\beta)\lambda(5\beta) + (2\beta)\lambda(3\beta) + (2\beta)\lambda(4\alpha) + (2\beta)\lambda(5\beta) + (3\beta)\lambda(4\alpha) + (3\beta)\lambda(5\beta) + (4\alpha)\lambda(5\beta) = A + 0 + 0 + 0 - A - B + 0 - A + 0 + A = -B^1 \quad (3)$$

$$\Sigma[\mu]_{D\,obs}^{20} \text{ of 1 C conformation} = (2\beta)\lambda(3\beta) + (2\beta)\lambda(4\beta) + (2\beta)\lambda(5\alpha) + (2\beta)\lambda(6\beta) + (3\beta)\lambda(4\beta) + (3\beta)\lambda(5\alpha) + (3\beta)\lambda(6\beta) + (4\beta)\lambda(5\alpha) + (4\beta)\lambda(6\beta) + (5\alpha)\lambda(6\beta) = -A + 0 + 0 + 0 + A - B + 0 + 0 + 0 + 0 = -B \quad (4)$$

Thus, $\Sigma[\mu]_{D\,obs}^{20}$ of (−)-1,2,3,5/4-cyclohexanepentol is always equal to $-B$ regardless of the conformation of the molecule. This is the reason why (−)-1,2,3,5/4-cyclohexanepentol is appropriate for the computation of ζ_{OH}^2. The value, $-B$, should of course be equal to the observed value of $[M]_D$(W), −8.9, of (−)-1,2,3,5/4-cyclohexanepentol. Therefore,

$$-B = -1.88\{(n^2+2)/3\}\,\zeta_{OH}^2 \equiv -8.9$$

$$\therefore\ \zeta_{OH}^2 = 4.7340\{3/(n^2+2)\} \quad (5)$$

Of course, the observed value, −8.9, is obtained at 27°C and not at 20°C. Strictly speaking, the value of ζ_{OH}^2 in Equation 5 is that for 27°C and not for 20°C.

On the other hand, however, when using the value of $[M]_D^{20}$(W) of (−)-1/2-cyclohexanediol[10], the value of ζ_{OH}^2 becomes as follows[11],

[3] N. A. B. WILSON and J. READ, J. chem. Soc. *1935*, 1269.

[4] TH. POSTERNAK and H. FRIEDLI, Helv. chim. Acta *36*, 251 (1953).

[5] B. MAGASANIK, R. E. FRANZL, and E. CHARGAFF, J. Am. chem. Soc. *74*, 2618 (1952).

[6] The orientations of the unit groups in 1 C conformation can be re-written in the bracket below by changing the locant numbers.

[7] S. YAMANA, Bull. chem. Soc. Japan *33*, 1741 (1960).

[8] L. P. KUHN, J. Am. chem. Soc. *74*, 2492 (1952); *76*, 4323 (1954).

[9] Given in the square brackets is the new value computed by using Equation 5.

[10] As it has two OH groups in *trans* orientation, (−)-1/2-cyclohexanediol is believed to exist, in water solution, almost perfectly in C 1 conformation (having two equatorial OH groups). See J. S. BRIMACOMBE, A. B. FOSTER, M. STACEY, and D. H. WHIFFEN, Tetrahedron *4*, 351 (1958).

[11] Tables I and II are used.

$\Sigma[\mu]^{20}_{D\,obs}$ of (−)-1/2-cyclohexanediol = $(1\beta)\lambda(2\alpha)$ = $-A = -11.73\{(n^2+2)/3\}\zeta^2_{OH} \equiv [M]^{20}_D(W)$ of (−)-1/2-cyclohexanediol, −54.0° $\therefore \zeta^2_{OH} = 4.6036\{3/(n^2+2)\}$ (6)

This value is nearly equal to the value in Equation 5. This fact indicates that the values in Equations 5 and 6 are both fairly reliable. The values of A and B for $[\mu]^{20}_{D\,obs}$ in the square brackets in the Note of Table II, can be obtained by multiplying those for $[\mu]^{20}_{D\,calcd}\{3/(n^2+2)\}$ by the value of $\zeta^2_{OH}\{(n^2+2)/3)\}$, 4.7340. Of course, when using Equation 5, new values of $\Sigma[\mu]^{20}_{D\,obs}$ of polyhydroxycyclohexanes of C 1 conformation which are shown in Table I of the previous paper[7] are apparently different from the observed values of $[M]_D(W)$. This fact can, however, be explained reasonably by the probable assumption that each of those polyhydroxycyclohexanes exists in the equilibrium mixture of C 1- and 1 C-conformers in its water solution. In this article, both of the series of values of A and B which are given in the Note of Table II, are used. When using these values in Equations 1 and 2, $\Sigma[\mu]^{20}_{D\,obs}$ of (−)-1,2/3,4-cyclohexanetetrol is calculated as 71.8 [or 93.2][9] (C 1 conformation and −141.7 [or −184.3][9] (1 C conformation). The observed value of $[M]^{20}_D(W)$, −109.2°, of (−)-1,2/3,4-cyclohexanetetrol indicates the presence of 15.3% [or 27.1%][9] of C 1 conformation and 84.7% [or 72.9%][8] of 1 C conformation in the equilibrium state. From these concentrations, one may calculate[12] the conformational equilibrium constant, K, and from this the free energy difference, $\Delta F°$, between the two conformations (i.e. the strength of the adjacent *trans* hydrogen bonding force between $(OH)^{2\beta}$- and $(OH)^{3\alpha}$- plus the difference in solvation energy between the solvent, water, and each of the two conformers) may be calculated as below:

$$K = [1\,C]/[C\,1] = 84.7/15.3 = 5.54$$
$$[\text{or, } K = 72.9/27.1 = 2.69]$$

$$\Delta F° = -RT\ln K = -2.303\,RT\log(5.54) = -1.0\ \text{kcal/mole}$$

$$[\text{or, } \Delta F° = -2.303\,RT\log(2.69) = -0.6\ \text{kcal/mole}]$$

This value seems to be comparable with −1.1 kcal/mole[13], the usual value of the corresponding hydrogen bonding force (O–H··O) in CCl_4 solution[8].

Zusammenfassung. Mit Hilfe der PM-Methode ist die molekulare Rotation von (−)-1,2/3,4-Cyclohexantetrol untersucht worden. Daraus geht hervor, dass diese Verbindung in wässeriger Lösung als Gemisch der C 1- und 1 C-Konstellationen vorliegt.

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Department of Chemistry, Kyoto Gakugei University, Fushimi-ku, Kyoto (Japan), January 5, 1965.

[12] R. A. PICKERING and C. C. PRICE, J. Am. chem. Soc. *80*, 4931 (1958).

[13] The $\Delta\nu$ of the *trans* hydrogen bond in 1/2-cyclohexanediol is 32 cm^{-1} [9], which corresponds to a $-\Delta H$ of 1.1 kcal/mole according to the JOESTEN-DRAGO equation[14] or a $-\Delta F$ of 1.1 kcal/mole, assuming $\Delta S \approx 0$.

[14] M. D. JOESTEN and R. S. DRAGO, J. Am. chem. Soc. *84*, 3817 (1962).

Chemical Structure of Circulin A

In the course of our work in the colistin[1] and polymyxin series[2] we investigated the structure of circulin A[3], another antibiotic exerting high activity against gram negative bacteria. KOFFLER and KOBAYASHI[4] deduced the chemical structure of this antibiotic tentatively as a cyclic decapeptide. This seemed to be very unusual in view of the close biological and chemical relationship between circulin A, the polymyxins and the colistins. The result of our re-examination now proves the structure of circulin A to be that shown in Figure 1[5], confirming that circulin A definitely belongs to the polymyxin family.

Circulin A produced by *Bacillus circulans* ATCC 14040 grown according to NELSON et al.[6] was isolated from the culture fluid mainly by ion exchange chromatography (Amberlite IRC-50, H^+-form) and CRAIG's counter-

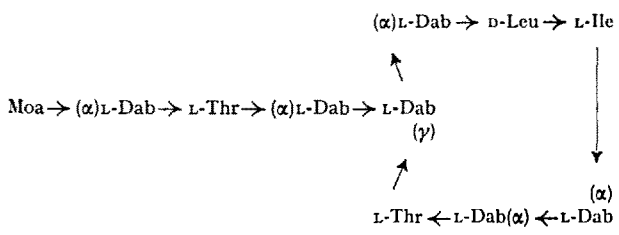

Fig. 1. Full structure for circulin A.

[1] T. SUZUKI, H. INOUYE, K. FUJIKAWA, and Y. SUKETA, J. Biochem. (Jap.) *54*, 25 (1963). – T. SUZUKI, H. INOUYE, K. FUJIKAWA, and S. NAGASAWA, J. Biochem. (Jap.) *54*, 173 (1963). – T. SUZUKI, K. HAYASHI, and K. FUJIKAWA, J. Biochem. (Jap.) *54*, 412 (1963). – T. SUZUKI and K. FUJIKAWA, J. Biochem. (Jap.) *56*, 182 (1964).

[2] T. SUZUKI, K. HAYASHI, K. FUJIKAWA, and K. TSUKAMOTO, J. Biochem. (Jap.) *56*, 335 (1964). – T. SUZUKI and K. HAYASHI, Abstr. of 2nd Symp. on *Peptide Chemistry*, Osaka, Japan (1963), p. 55.

[3] F. J. MURRAY and P. A. TETRAULT, Proc. Soc. Am. Bact. *1*, 20 (1948). – F. J. MURRAY, P. A. TETRAULT, O. W. KAUFMANN, H. KOFFLER, D. H. PETERSON, and D. R. COLINGSWORTH, J. Bact. *57*, 305 (1949). – D. H. PETERSON and L. N. REINEKE, J. biol. Chem. *181*, 95 (1949). – J. H. DOWLING, H. KOFFLER, H. C. REITZ, D. H. PETERSON, and P. A. TETRAULT, Science *116*, 147 (1952).

[4] H. KOFFLER and T. KOBAYASHI, Abstr. Div. Biol. Chem. Ann. Chem. Soc., April 13–18 (1958), San Francisco.

[5] The following abbreviations are used throughout this paper: Moa = (+)-6-methyloctanoyl residue; Dab = α,γ-diaminobutyric acid residue; DNP = 2,4-dinitrophenyl residue; → = C to N bond in –CO–NH–; →(α) Dab = →Dab; →(γ) Dab = →Dab. (γ-NH_2; α-NH_2)

[6] H. A. NELSON, C. DEBOER, and W. H. DEVRIES, Ind. Eng. Chem. *42*, 1259 (1950).