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PREPARATION AND PERIODATE OXIDATION OF C-6-OXYCELLULOSE: CONFORMATIONAL INTERPRETATION OF HEMIACETAL STABILITY*

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ABSTRACT

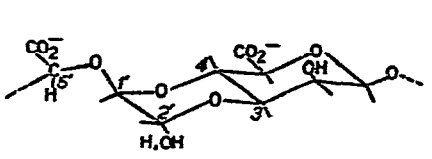
After correction for overoxidation, the periodate-oxidation limit of the soluble, sodium salt of C-6-oxycellulose was 0.63 mol per non-terminal, hexuronic acid residue, compared to 0.44 mol for a (1→4)-linked β-D-mannuronan isolated from alginate. This suggested that, whereas both the aldehyde groups of oxidised hexuronic acid residues formed stable inter-residue hemiacetals in the mannuronan, only the one derived from C-2 did this in the glucuronan. Formation of the other hemiacetal in the oxidised glucuronan would require C-5 of the oxidised residue to become axial with respect to the resultant 1,4-dioxane ring, and the p orbitals of O-1 and O-5 in the adjacent unoxidised residue to become eclipsed. The extent of the destabilisation suggests that both conditions have to be recognised as important instability factors in this kind of hemiacetal.

INTRODUCTION

The anomalous periodate-oxidation limit of alginates is consistently 0.44 mol per non-terminal hexuronic-acid residue, and corresponds to the theoretical value expected for spontaneous formation of the stable hemiacetals **1** and **2** by the aldehyde groups originating from C′-2 and C′-3 of oxidised residues, respectively[1]. For the corresponding β-D-glucuronan (C-6-oxycellulose), one would expect the hemiacetal **3** formed by C′-2 of oxidised residues to be just as stable as **1**, whereas the one (**4**) formed by C′-3 would encounter steric and electronic interactions that are absent in **2**.

Experimental investigation of this point was complicated by the impossibility of oxidising cellulose quantitatively at C-6 without depolymerising it sufficiently to cause serious overoxidation in the subsequent treatment with periodate. Overoxidation could not be adequately suppressed by the careful control[2] of pH or temperature[3], but a way was found to correct for it, and a result was obtained that indicated two important instability factors in this kind of hemiacetal.

*Dedicated to the memory of Prof. J. K. N. Jones, F.R.S.



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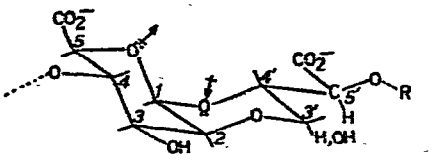

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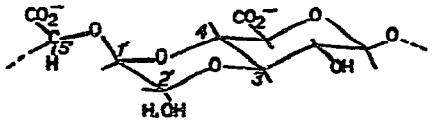

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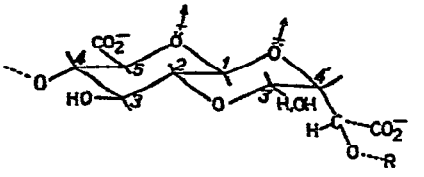

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EXPERIMENTAL

Materials and analytical methods. — The β-D-mannuronan was isolated by fractionation of the products of mild acid-hydrolysis of alginate isolated from young fronds of *Laminaria digitata*[4,5]. It had a ManA/GulA ratio[6] of 13.5, corresponding to 94% of D-mannuronic acid residues, and a reducing equivalent[7] corresponding to a number-average degree of polymerisation (d.p.$_n$) of 48. Because of the presence of firmly-bound moisture[8], the dry weight of the sodium salt was determined by titration with cetylpyridinium chloride[1,9].

C-6-Oxycellulose was dried to constant weight over phosphorus pentaoxide at 80°. Its equivalent weight was determined by titration with 0.01M sodium hydroxide, by using phenolphthalein as indicator. D-Glucuronic acid was determined by a modification[10] of the Dische carbazole method, with D-glucuronolactone as standard.

Periodate oxidation was performed as described earlier[1]. The mixtures were made 2M with respect to sodium chloride (to quench the Donnan-equilibrium effect[1]), and were initially neutralised to methyl red. During the reaction, the pH decreased to about 2.8 because of the liberation of formic acid.

Preparation of C-6-oxycellulose. — Cotton wool (5 g) was dissolved in ortho-phosphoric acid (85% w/w; 100 ml) by vigorous grinding in a large (~1 litre) mortar for 2 h at 20°. Sodium nitrite (5 g), which had previously been ground to a very fine powder, was added portionwise, with vigorous mixing, over a period of 30 min. The mixture was kept in the fume hood for 5 h, without disturbing the white foam that expanded until it almost filled the mortar. The foam was then broken down with the pestle, and a further portion (5 g) of sodium nitrite was dispersed in the liquid. After a further 3 h, this addition was repeated, and after a total time of ~12 h, formic acid (90% w/w; 5 ml) was added to reduce the excess of N_2O_3. Ice-cold ether (300 ml) was then added cautiously, with vigorous mixing, in a fume hood, and with skin- and

eye-protection. The white precipitate was collected and extracted repeatedly by grinding it with ether. It was then washed exhaustively with distilled water until the washings were neutral, and dried by solvent-exchange with ethanol and ether. The yield of pure-white solid was 3.75 g. This was added portionwise to aqueous sodium borohydride (20% w/v; 50 ml), with stirring until it had dissolved. After 24 h, the solution was neutralised with acetic acid, dialysed against distilled water, and freeze-dried.

RESULTS

Improved preparation of C-6-oxycellulose. — Commercial and laboratory samples, prepared by exposing solid cellulose to nitrogen dioxide[11], were yellow to brown in color, contained nitrogen, and were highly degraded, as indicated by the low intrinsic viscosities of their sodium salts. Despite the attendant acid-hydrolysis, a cleaner and less-degraded product was obtained by adding finely-powdered sodium nitrite to a homogeneous solution of cellulose in syrupy phosphoric acid. The active oxidant in the reaction was probably N_2O_3, as the success of the method depended upon the production and maintenance of a stable foam within the viscous solution.

The initial product had an equivalent weight of 184 and contained 87.5% of D-glucuronic acid. A treatment with aqueous sodium borohydride increased the latter figure to 93.5%, probably by reducing 2- and 3-keto groups. The last figure agrees quite well with the equivalent weight, but may be too low if the glucuronan shared the the resistance of alginates to complete drying[8].

Attempts to determine the molecular weight of the material by chemical methods were initially frustrated by uncertainty as to whether the N_2O_3 had cleaved the carbon chains of the end-groups. Periodate oxidation of the borohydride-reduced material was nevertheless performed at 0° (to suppress overoxidation[3]), and it led to an initial, rapid uptake of 0.098 mol of oxidant, with release of 0.033 mol of formic acid (Fig. 1). This suggested a $d.p._n$ of ~66, on the assumption that the reducing end-groups had been converted into D-glucaric acid, and the non-reducing ones into D-glucuronic acid*. This estimate was supported by the finding that the intrinsic viscosity of the material (0.92 dl/g in 0.1M sodium chloride) was similar to that of alginate fragments of this size.

Correction for periodate overoxidation. — Although it was successful when applied to fragments of alginate[1], the usual method of correction, consisting in back-extrapolation of the terminal part of the reaction curve to zero time, failed with C-6-oxycellulose. This was not because the overoxidation was faster (which would not be expected), but because Malapradian oxidation of the main chain was much slower. The two kinds of oxidation were consequently similar in rate, and it was impossible to detect any discontinuity in the reaction curve.

*All calculations in this paper are based upon the fact[12] that hexopyranosiduronates rapidly consume not two, but three moles of periodate. The third mole is consumed in hydroxylating at C-5, to give a hemiacetal[12] whose further oxidation is probably retarded by formation of a cyclic hemiacetal with the aldehyde group derived from C-2.

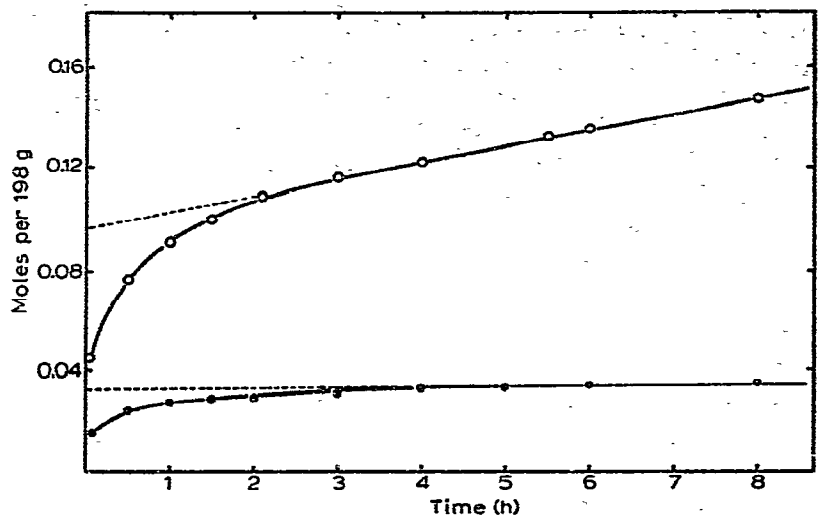


Fig. 1. Consumption of periodate (○) and release of formic acid (●) in the initial stages of the oxidation of 10mM C-6-oxycellulose (Na^+ salt) in 12.5mM sodium metaperiodate at 0°. The reaction mixture was 2M with respect to sodium chloride.

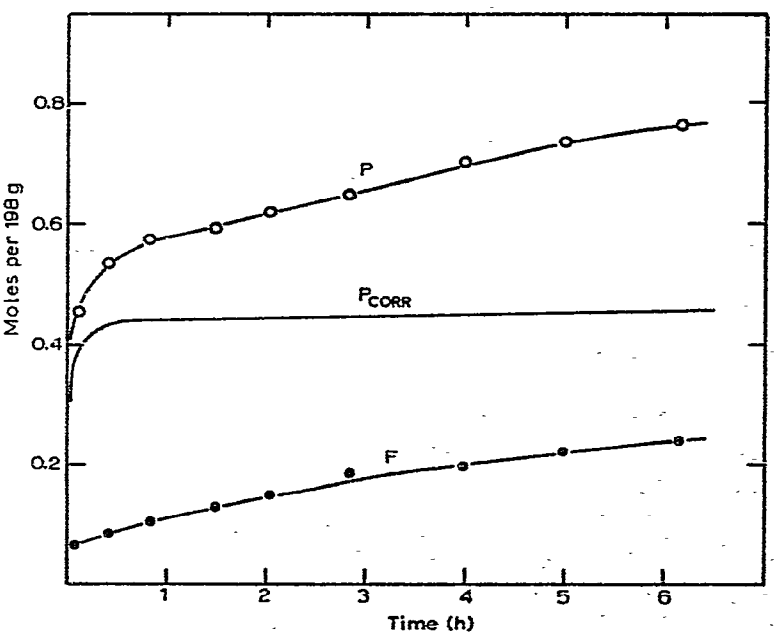


Fig. 2. Consumption of periodate (P) and release of formic acid (F) by 8mM D-mannuronan (Na^+ salt) in 25mM sodium metaperiodate at 25°. The reaction mixture was 2M with respect to sodium chloride. P_{corr} was calculated from the formula $(P-0.108)-1.4(F-0.065)$.

It was therefore necessary to abandon any attempt to suppress overoxidation, and to try instead to correct for it. This was done by following the release of formic acid (F), in excess of that originating from end-groups (Fig. 1), and using the result to calculate the amount of periodate that had been consumed in bringing about overoxidation.

Consideration of accepted mechanisms[2,12] suggested that this amount would be $1.25F$, both for the familiar kind of overoxidation that takes place from the reducing end, and for the kind that, in glycuronans, also takes place from the non-reducing end[12]. It was, however, also necessary to take account of the fact that the stepwise "peeling" of the chain would lead to the exposure of intact hexuronic acid residues, whose oxidation would otherwise have been prevented by inter-residue hemiacetal formation. For these residues, the correction would be $1.50F$.

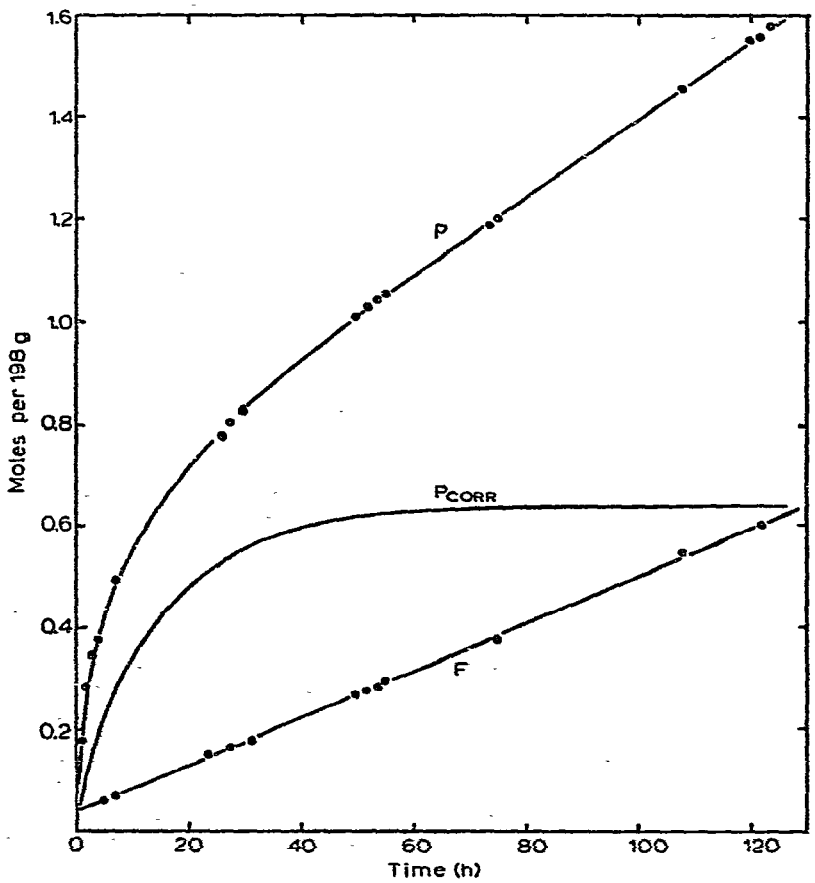


Fig. 3. Consumption of periodate (P) and release of formic acid (F) by 10mM C-6-oxycellulose (Na^+ salt) in 25mM sodium metaperiodate at 25°. The mixture was 2M with respect to sodium chloride. P_{corr} was calculated from the formula $(P-0.098)-1.34(F-0.033)$.

To test the validity of this method, an experiment was conducted with a fragment of alginate having a d.p.$_n$ of 48, and consisting almost entirely of D-mannuronic acid residues. As it was known[1] that this material would consume only 0.44 mol of periodate per non-terminal residue in the Malapradian manner, the correction should be $(0.44 \times 1.25)F + (0.56 \times 1.50)F = 1.40F$.

The results (Fig. 2) indicated an initial, rapid liberation of 0.065 mol of formic acid, which would have resulted from the consumption of 0.108 mol (that is, $0.065 \times 5/3$ mol) of periodate by the two end-groups. The curve for P_{corr} in Fig. 2 was calculated from the formula $P_{corr} = (P - 0.108) - 1.4(F - 0.065)$, and it indicates an anomalous oxidation-limit of 0.44 mol, in exact agreement with theory.

Measurement of the Malapradian oxidation-limit of C-6-oxycellulose. — The results are shown in Fig. 3. The curve for P_{corr} was calculated from the formula $(P - 0.098) - 1.34(F - 0.033)$, the correction for end-group effects being based upon the results shown in Fig. 1. The factor of 1.34, equal to $(0.64 \times 1.25) + (0.36 \times 1.50)$, was chosen when it became clear that the limit would be near to 0.64 mol. A systematic approach would be arbitrarily to choose a factor of 1.25 in the first instance, and to obtain an approximate value for the limit by extrapolating the linear part of the curve for P_{corr} to zero time. The limiting value of P_{corr} in Fig. 3 is 0.63 ± 0.01 mol.

DISCUSSION

There can be little doubt that the oxidation-limit of C-6-oxycellulose is just as "anomalous" as that of alginate, in the sense that Malapradian oxidation of the main chain becomes too slow to measure before the theoretical limit of 1.0 mol per non-terminal residue is reached. This is not surprising, because OH-2 in **1** and **3** could not interact sterically with C'-1 or C'-2 in the hemiacetal ring, and these hemiacetals are otherwise identical.

It is also clear that the oxidation-limit is considerably higher than that of alginate. Because of the possible presence of up to 6% of D-glucose residues in the experimental material, the measured oxidation-limit of 0.63 mol may be a little higher than that of a pure β-D-glucuronan, but it cannot be higher by more than 0.022 mol (that is, 6% of 0.37 mol). It is therefore certain that the oxidation-limit of a homopolymeric D-glucuronan must be at least 0.60 mol per non-terminal residue. Calculations[13,14] have shown that this is the limit that would be expected, if one aldehyde group spent all its time as an inter-residue hemiacetal, while the other spent only half its time in the hemiacetal form. This provides an indication of the stability of **4**.

We have not yet succeeded in obtaining a similar estimate of the stability of **2**. Periodate-oxidised alginates of high molecular weight do not even react with Schiff's reagent[1]. The terminal rate of oxidation of alginate is too low to be measured accurately, but is it certainly not more than 0.1% of the initial rate. This suggests a minimal value for the stability of **2**, and implies that the free-energy change that is

associated with ring-closure must be *at least* 4 kcal.mol^{-1} more favorable in **2** than it is in **4**.

Ring-closure entails entropic as well as enthalpic changes, but the problem of interpretation is greatly simplified by the fact that, in the open-chain forms of **2** and **4**, the acyclic parts are configurationally identical. Moreover, in both isomers, C′-4 would be likely to spend most of its time *anti*-periplanar to C-2 of the glycose ring*, and in that conformation, the configuration of OH-2 would have no effect upon the rest of the chain. It is, therefore, a reasonable approximation to assume that, in the open-chain forms, the acyclic parts are also conformationally identical.

It is now easy to recognise that ring-closure would introduce just two instability factors into **4** that it would not introduce into **2**: (*a*) the axial conformation of C′-5, and (*b*) the eclipsed p-orbitals of O-1 and O-5. On the other hand, it would introduce one interaction into **2** that is not found in **4**: (*c*) the *gauche* interaction between C′-4 and O-5.

We would point out that factor (*a*) can be formally considered to take account of any solvational changes associated with the erection of the $-CH(OR)CO_2^-$ group to an axial position, whereas (*b*) would similarly take account of any changes in the solvation of O-1 and O-5 which ring-closure brings about in one isomer, but not in the other. Ring-closure must also entail important changes in the solvation of O-2 and the aldehyde group derived from C′-3, but it is reasonable to expect that these would be identical in the two isomers**.

Interestingly, unlike *cis*-decalin, structure **2** contains no *inter*-annular interactions between axial hydrogen atoms (the so-called "H-inside" condition). It would also not be significantly stabilised by entropy of mixing, because the $^4C_1$ conformation of β-D-mannopyranuronic acid is too stable, and the other hemiacetal (**1**), when it is present, would in any case preclude the alternative conformer.

For the familiar interaction (*c*), it should be safe to accept Angyal's well-tested figure[19] of 0.35 kcal.mol^{-1}, although the shortness of the C-1–O-1 bond may mean that it is, in this case, a little too low. Insofar as they occur in 1,4-dioxane rings, there are no precedents for the instability factors (*a*) and (*b*), but the present experimental result may be readily explained, if:

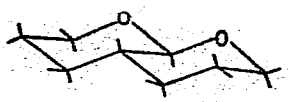
(*a*) The $-CH(OR)CO_2^-$ group is compared to the isopropyl group, for which conformational energies, in cyclohexane rings, of up to 3.55 kcal.mol^{-1} have been reported[20].

(*b*) The electronic interaction is similar in magnitude to that investigated by Descotes *et al.*[21], who measured the position of the equilibrium between **5** and **6** in aqueous acid at room temperature. After correcting for steric interactions and the

*This refers to the steric preference, together with the *exo*-anomeric effect[15,16]. In the solid state, C′-4 takes up this conformation in both cellulose[17] and alginate[18].

**We are, of course, only concerned with *changes* that accompany ring-closure. It is likely that O-2 will be more highly solvated when it is equatorial than when it is axial, but our assumption is that this will still be true after ring-closure.

entropy of mixing of the *cis*-isomer, a value of 1.4 kcal.mol^{-1} was obtained for the electronic effect alone[21].



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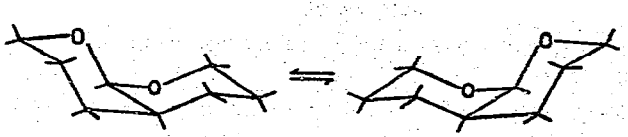

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By studying a sufficient number of other polysaccharides, it should be possible to arrive at independent values for (*a*) and (*b*), and to rationalise the stabilities of other hemiacetals, such as **1** and **3**. For example, the fact that β-(1→4)-linked xylans are completely oxidisable by periodate[22,23] makes it easy to recognise that the stabilities of **1** and **3** are due largely to the interaction between C′-1 of the oxidised unit and the carboxylate anion of the intact hexuronic-acid residue. This interaction would virtually preclude the rotamer in which C′-1 is *anti*-periplanar with C-3 of the glycose ring, whereas, in xylan, this would be the most stable rotamer.

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REFERENCES

1 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 813–833.
2 L. HOUGH, T. J. TAYLOR, G. H. THOMAS, AND B. WOODS, *J. Chem. Soc.*, (1958) 1212–1217.
3 A. L. POTTER AND W. Z. HASSID, *J. Am. Chem. Soc.*, 70 (1948) 3488–3490.
4 A. HAUG, B. LARSEN, AND O. SMIDSRØD, *Acta Chem. Scand.*, 21 (1967) 691–704.
5 A. HAUG, B. LARSEN, AND O. SMIDSRØD, *Carbohydr. Res.*, 32 (1974) 217–225.
6 A. HAUG AND B. LARSEN, *Acta Chem. Scand.*, 16 (1962) 1908–1918.
7 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
8 E. PERCIVAL AND R. H. MCDOWELL, *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London and New York, 1967, 1–219, see pp. 106 and 114.
9 J. E. SCOTT, *Methods Biochem. Anal.*, 8 (1960) 146–195, 163.
10 T. BITTER AND H. M. MUIR, *Anal. Biochem.*, 4 (1962) 330–334.
11 T. P. NEVELL, *Methods Carbohydr. Chem.*, 3 (1963) 164–185.

12 M. CANTLEY, L. HOUGH, AND A. O. PITTET, *J. Chem. Soc.*, (1963) 2527–2535.
13 O. SMIDSRØD, B. LARSEN, AND T. J. PAINTER, *Acta Chem. Scand.*, 24 (1970) 3201–3212.
14 J. J. GONZÁLEZ, P. C. HEMMER, AND J. S. HØYE, *Chem. Phys.*, 3 (1974) 228–238.
15 R. U. LEMIEUX, in P. DE MAYO (Ed.), *Molecular Rearrangements*, Vol. 2, Interscience, New York, London and Sydney, 1964, 709–769, p. 738.
16 R. U. LEMIEUX AND J. C. MARTIN, *Carbohydr. Res.*, 13 (1970) 139–161.
17 R. H. MARCHESSAULT AND A. SARKO, *Adv. Carbohydr. Chem.*, 22 (1967) 421–482, see p. 439.
18 E. D. T. ATKINS, W. MACKIE, I. A. NIEDUSZYNSKI, K. D. PARKER, AND E. E. SMOLKO, *Biopolymers*, 12 (1973) 1865–1878.
19 E. L. ELIEL, N. L. ALLINGER, S. J. ANGYAL, AND G. A. MORRISON, *Conformational Analysis*, Interscience, New York, London and Sydney, 1965, 1–487, p. 356.
20 See ref. 19, p. 440.
21 G. DESCOTES, M. LISSAC, J. DELMAU, AND J. DUPLAN, *Compt. Rend.*, 267C (1968) 1240–1241.
22 S. K. CHANDA, E. L. HIRST, J. K. N. JONES, AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1950) 1289–1297.
23 T. J. PAINTER AND B. LARSEN, *Acta Chem. Scand.*, 24 (1970) 2366–2378.