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INCLUSION OF SPIRONOLACTONE IN CYCLOMALTOHEPTAOSE: A GUEST AFFECTED BY THE HOSPITALITY OF THE HOST

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ABSTRACT

A study of the interaction of spironolactone (SP) and cyclomaltoheptaose (β-cyclodextrin, βCD), using molecular graphics, shows a possible stable 1:2 SP/βCD inclusion complex. The complex was prepared by coprecipitation from an aqueous solution and inclusion was confirmed in the solution and solid state, using the solubility technique, i.r. spectroscopy, and d.s.c. N.m.r. spectroscopy revealed chemical modification (*S*-deacetylation) of SP due to the environment of the βCD.

INTRODUCTION

In the field of pharmaceutical technology, inclusion with cyclomalto-oligosaccharides (cyclodextrins, CDs) has been applied extensively in order to enhance chemical or physical stability, solubility, rate of dissolution, and bioavailability of slightly soluble drugs[1]. The formation of inclusion complexes is equivalent to molecular encapsulation and requires favourable steric and electrostatic interaction of the host and guest molecules. Molecular graphics allow an investigation of possible host–guest stoichiometries and visualization of molecular adaptation, which enables the design of hosts of optimum size for inclusion. Intra- and intermolecular effects, which can also be examined for highly reactive groups, may lead to chemical modification of the host or guest. However, this approach should always be supported by experimental evidence such as n.m.r. data. We now report on the inclusion of spironolactone by βCD.

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EXPERIMENTAL

General. — βCD was supplied by Roquette Frères (France), spironolactone was purchased from Coopérative Pharmaceutique Française (France), and $(CD_3)_2SO$ was obtained from the CEA (Saclay, France).

U.v. and i.r. spectra were recorded with Pye Unicam 1700 and SP 3200 spectrophotometers, respectively. A Du Pont de Nemours DSC990 differential scanning calorimeter was used for thermal analysis. N.m.r. spectra (500 MHz for ^1H, 75.47 MHz for ^{13}C) were obtained with Bruker WM500 and MSL300 spectrometers, respectively. The SYBYL 5,10 program[2] and an Evans and Sutherland PS300 high-resolution graphics system controlled by a VAX computer were used for the molecular graphics study.

Phase solubility studies. — Solubility measurements were carried out using the method reported by Higuchi and Connors[3]. Excess of SP was shaken with aqueous βCD of various concentrations at 37 ±0.5°. After 7 days, an aliquot was centrifuged and filtered, and a portion was diluted with aqueous 50% ethanol and analyzed spectrophotometrically at the λ_{max} (241 nm) of spironolactone. The phase solubility diagram (Fig. 1) shows a typical B_s type solubility curve[4,5].

Preparation of the solid inclusion complex. — The conditions used were derived from Fig. 1, and the amounts were calculated from the descending curve. Typically, a mixture of SP (0.21 g) and βCD (2.84 g) was added to water (100 mL), sealed in a flask, and stirred for 4 days at 37°. After decantation (2 days), the precipitate was collected, and dried *in vacuo* over phosphorus pentaoxide.

Solubility and rates of dissolution. — An excess of the solid was stirred with water (10 mL) for 7 days at 37°. The mixture was centrifuged, and the supernatant solution was diluted and analyzed spectrophotometrically as above. A dispersion of the sample in water (300 mL) at 37 ±0.5° was stirred with a paddle at 50 $r.min^{-1}$.

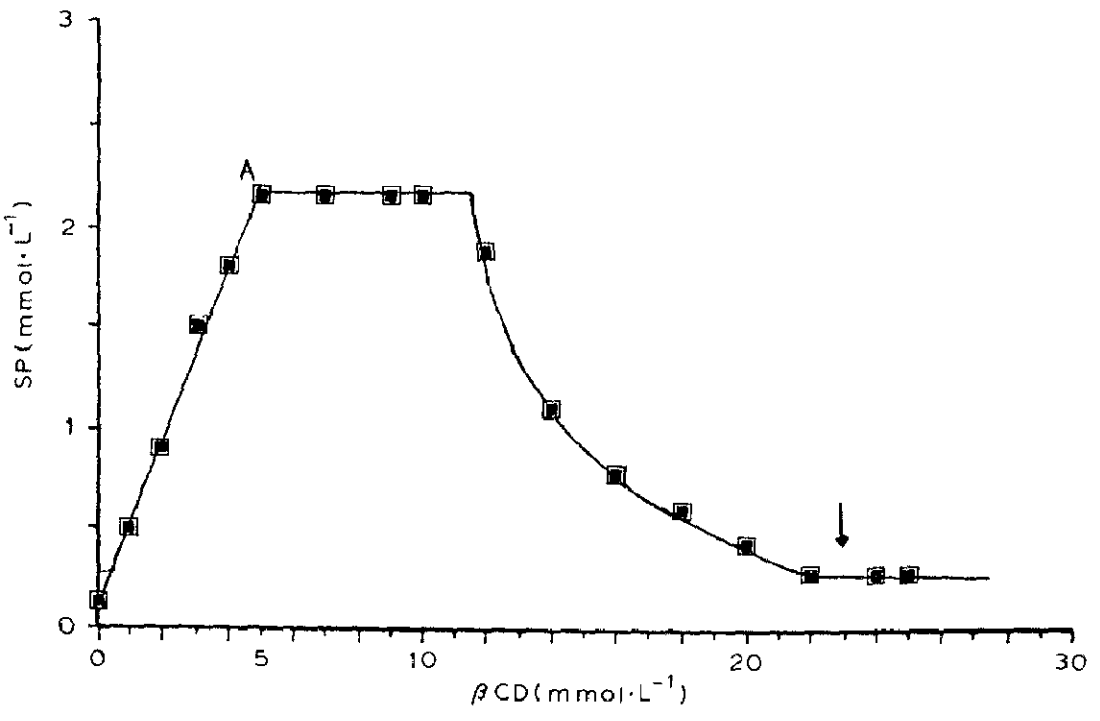

Fig. 1. Phase solubility diagram of the SP/βCD system in water at 37°.

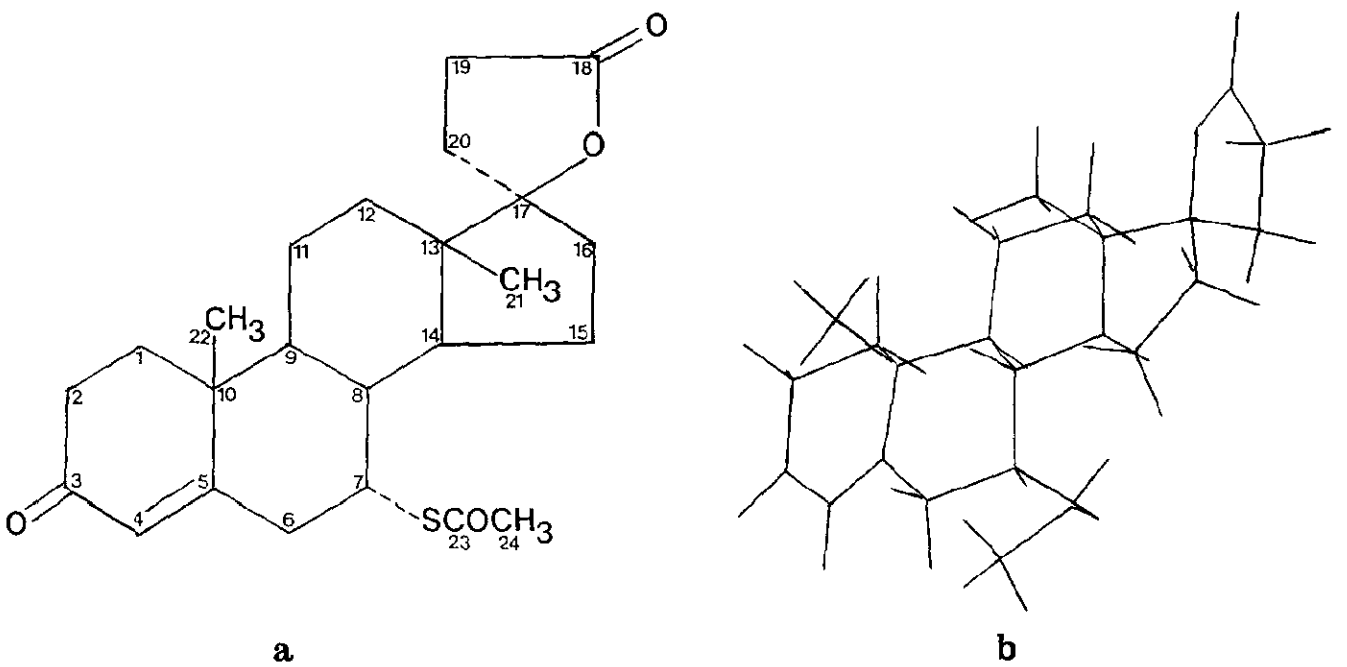

Fig. 2. Structures of (a) the SP molecule and (b) the energetically optimized SP molecule.

The apparatus (similar to the USP XX paddle apparatus) was connected to the spectrophotometer by a peristaltic pump and the rates of dissolution were then monitored automatically.

RESULTS AND DISCUSSION

Molecular graphics of SP and βCD. — The use of molecular graphics in the design of pharmaceutical products and their interactions with receptor sites is a rapidly expanding area of research. We have applied this technique to the formation of inclusion complexes by CDs with special reference to the selectivity of the interactions with chiral substances, and their possible rôle in the separation of enantiomers.

Molecular modelling of the SP/βCD inclusion complex was carried out using the SYBYL molecular graphics program. There are considerable limitations on the model of any CD inclusion complex derived from molecular graphics. For example, hydrophobic–hydrophilic interactions and dynamic and solvent effects are not considered, and no account is taken of the large supra-molecular assemblies of βCD in solution. In view of these constraints, the studies were limited to a determination of the most probable stoichiometry and positioning of the SP molecule (see Fig. 2a) in the cavity of the βCD.

The SP molecule was constructed via the Building sub-routine, using androsterone as the molecular template. The required atoms and fragments were added to this molecule, and the target molecule was energy-minimized using the OPTIMIZATION sub-routine with the SYBYL MAXIMIN-2 program. The minimization was carried out first over 50 cycles with no partial charges, and hence no electrostatic contribution, and then repeated using electrostatic contributions and charges as generated by SYBYL (DELRE). The differences in geometry from

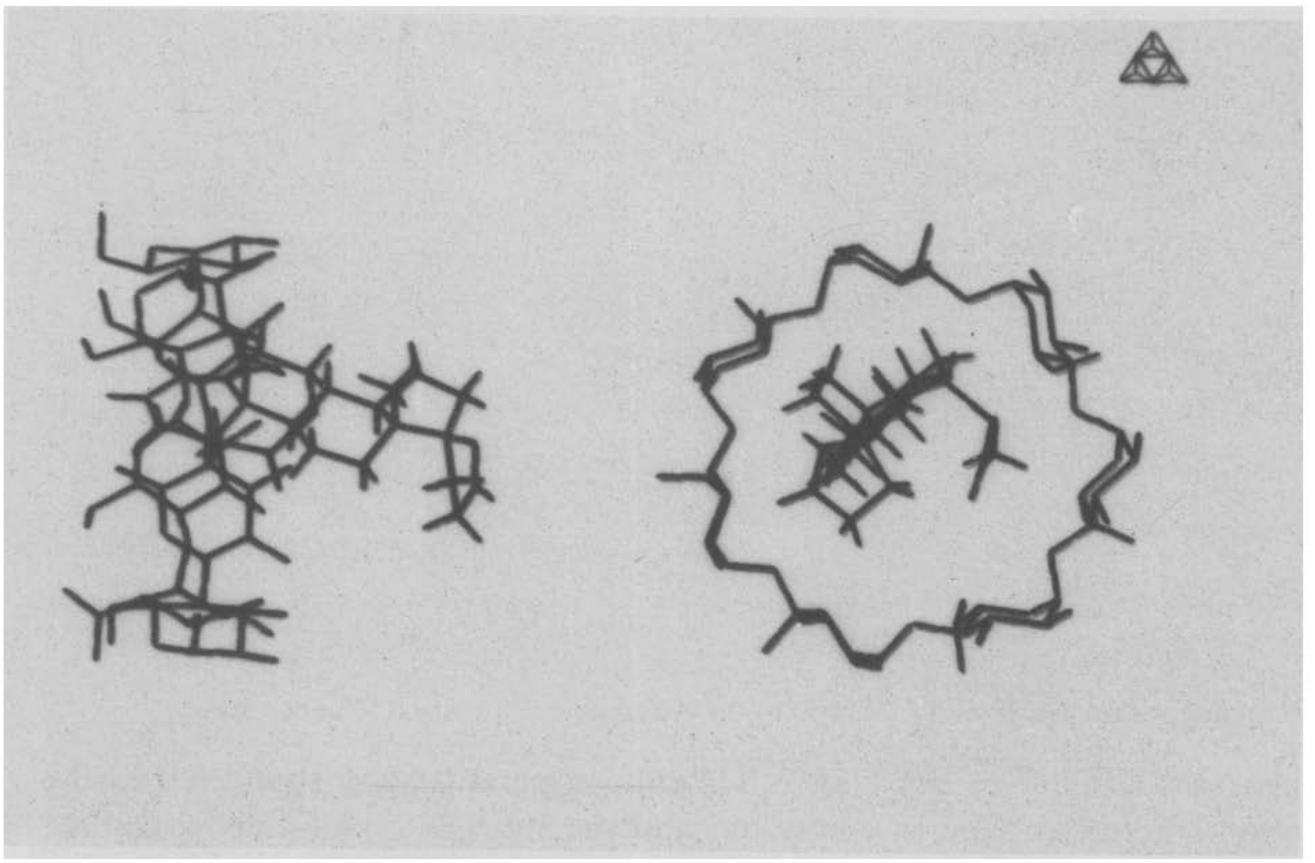

Fig. 3. Molecular graphics model of the SP/βCD inclusion compound.

Fig. 4. Molecular graphics model of the SP/$(\beta CD)_2$ inclusion compound.

the two calculations were minimal (<0.1 Å). Since, in the docking model, the use of electrostatic interactions would require dynamic analysis of the complex and its solvent environment, the non-electrostatic model was used (Fig. 2b).

The model for the SP/βCD complex was obtained using manual docking with the screen in the ORTHOGONAL mode. The 1:1 inclusion complex leaves ~40% of the hydrophobic SP molecule outside the cavity of the βCD. Fig. 3 shows the complex after minimization. An alternative model was also considered comprising a $(\beta CD)_2$/SP complex (Fig. 4). Several βCD inclusion compounds[6] have a dimeric structure in the solid state with the secondary faces of the βCD molecules bound by hydrogen bonds. At this point, the O-2/O-2′ and O-3/O-3′ distances were set to 2.7 Å as found in the benzyl and benzophenone dimeric βCD inclusion complexes[6].

Energy minimization was carried out to verify that no large repulsive van der Waals interactions of the host and guest were present. Subsequent energy minimization of $(\beta CD)_2$/SP showed van der Waals energies of -15 kcal.mol^{-1}, *i.e.*, an energetically favourable situation, relative to the sum of separately calculated van der Waals energies of SP and the βCD dimer, whereas the van der Waals energy of the SP/βCD complex was -5 kcal.mol^{-1}. Based on these results, it was possible to predict that a $(\beta CD)_2$/SP complex should be stable. As it is extremely difficult to take into account the dynamic nature of the system, the effects of hydrophobic–hydrophilic interactions have not been studied, and, in consequence, the energy differences should be regarded as qualitative and not quantitative.

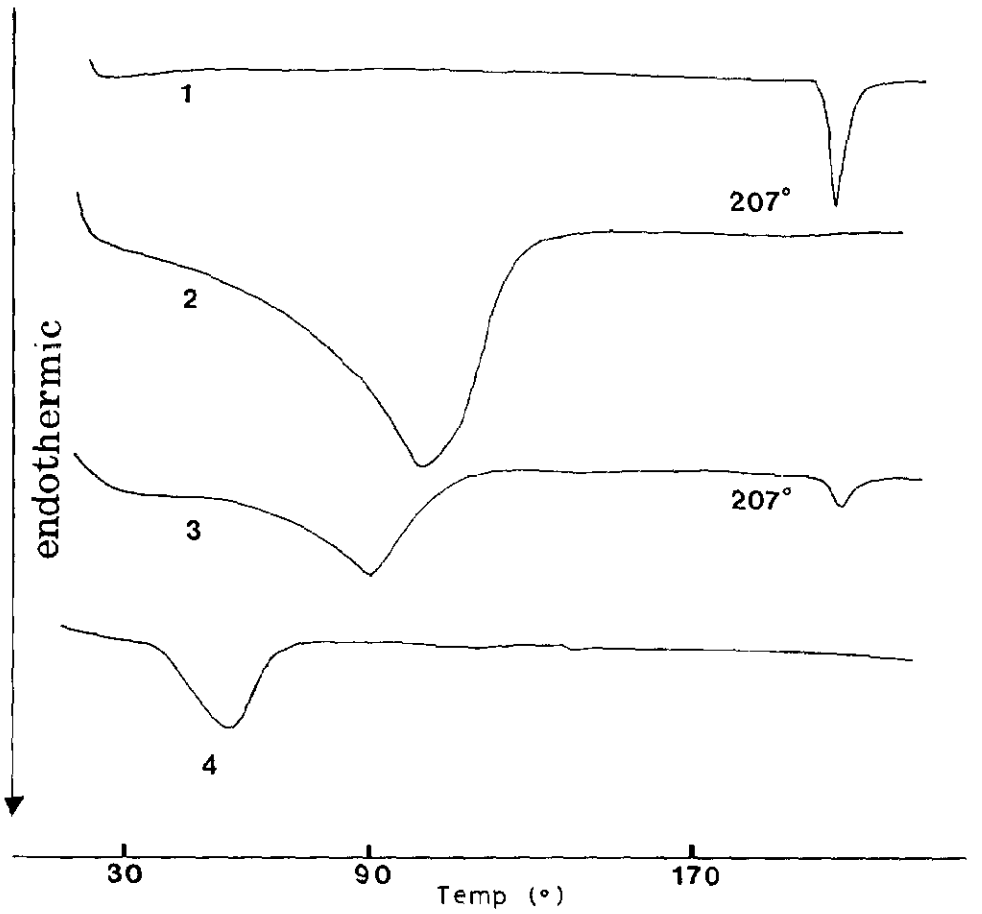

Fig. 5. D.s.c. of (1) SP, (2) βCD, (3) a physical mixture of SP and βCD, and (4) the SP/βCD inclusion compound.

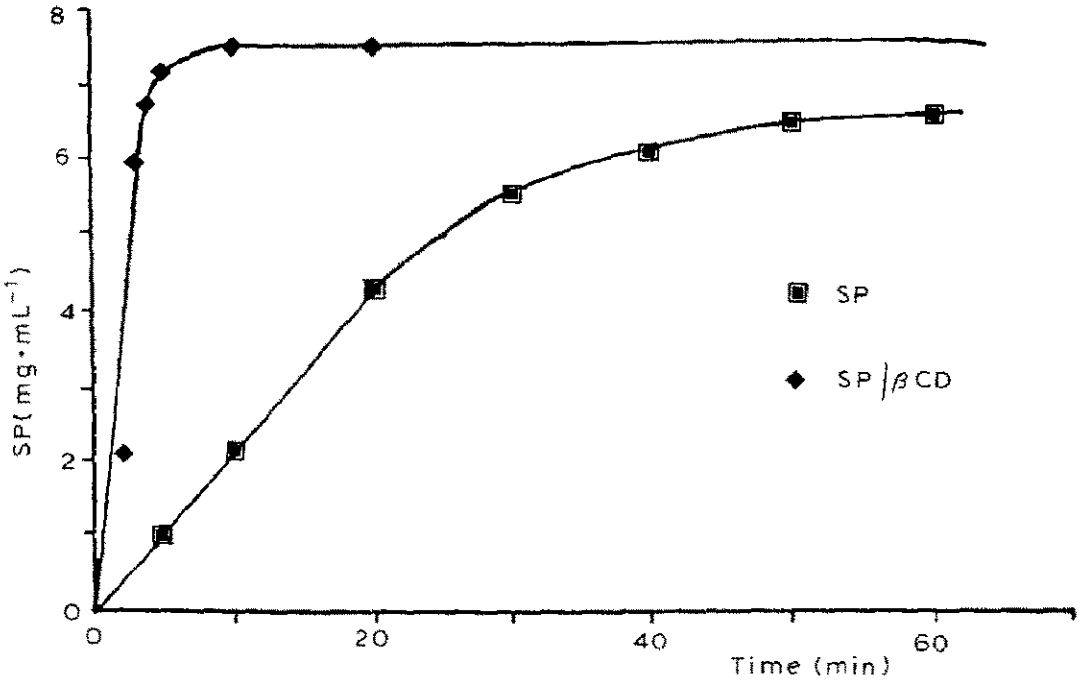

Fig. 6. Dissolution profiles of SP and the SP/βCD inclusion complex in water at 37°.

Inclusion compound in the solid state. — The i.r. spectra of SP and its physical mixture with βCD were characterized by two carbonyl bands at 1670 and 1770 cm^{-1}. However, in the spectrum of the inclusion complex, these bands were much weaker, which may reflect restriction effects in the cavity and chemical modification (see below) involving the carbonyl groups.

D.s.c. showed clearly that the complex exists in the solid state as reflected by the thermograms in Fig. 5. As expected, the endothermic peak of SP was not shown by the inclusion complex.

Fig. 6 shows dissolution profiles for SP and the inclusion complex. The rate of dissolution of the latter was significantly faster, and its solubility in water was higher (26 mg 100 mL^{-1} versus 4.5 mg 100 mL^{-1} for SP).

The above results show clearly the existence of an SP/βCD complex both in aqueous solution and in the solid state.

N.m.r. spectroscopy. — N.m.r. experiments have been used to investigate the stoichiometries of inclusion complexes[7], to prove the existence of complexes in solution[8,9], and to determine the approximate geometry of the guest within the cavity of the CD using n.O.e. effects[10]. However, the generally low aqueous solubility of inclusion complexes derived from βCD has limited these studies.

N.m.r. experiments performed on solutions in $(CD_3)_2SO$ are restricted mainly to the determination of stoichiometries and of potential chemical modifications, since release of the host molecule occurs due to the strong solvation effects. Comparison of the n.m.r. solution spectra of the SP/βCD complex and βCD in $(CD_3)_2SO$ did not show any meaningful difference. However, for dilute solutions (Fig. 7) in D_2O, the resonances of H-3 and H-5 in the complex were shifted upfield markedly, indicating the presence of a stable complex. The 500-MHz n.m.r. spectra of SP and the disassociated complex in Me_2SO are shown in Fig. 8. The assignment

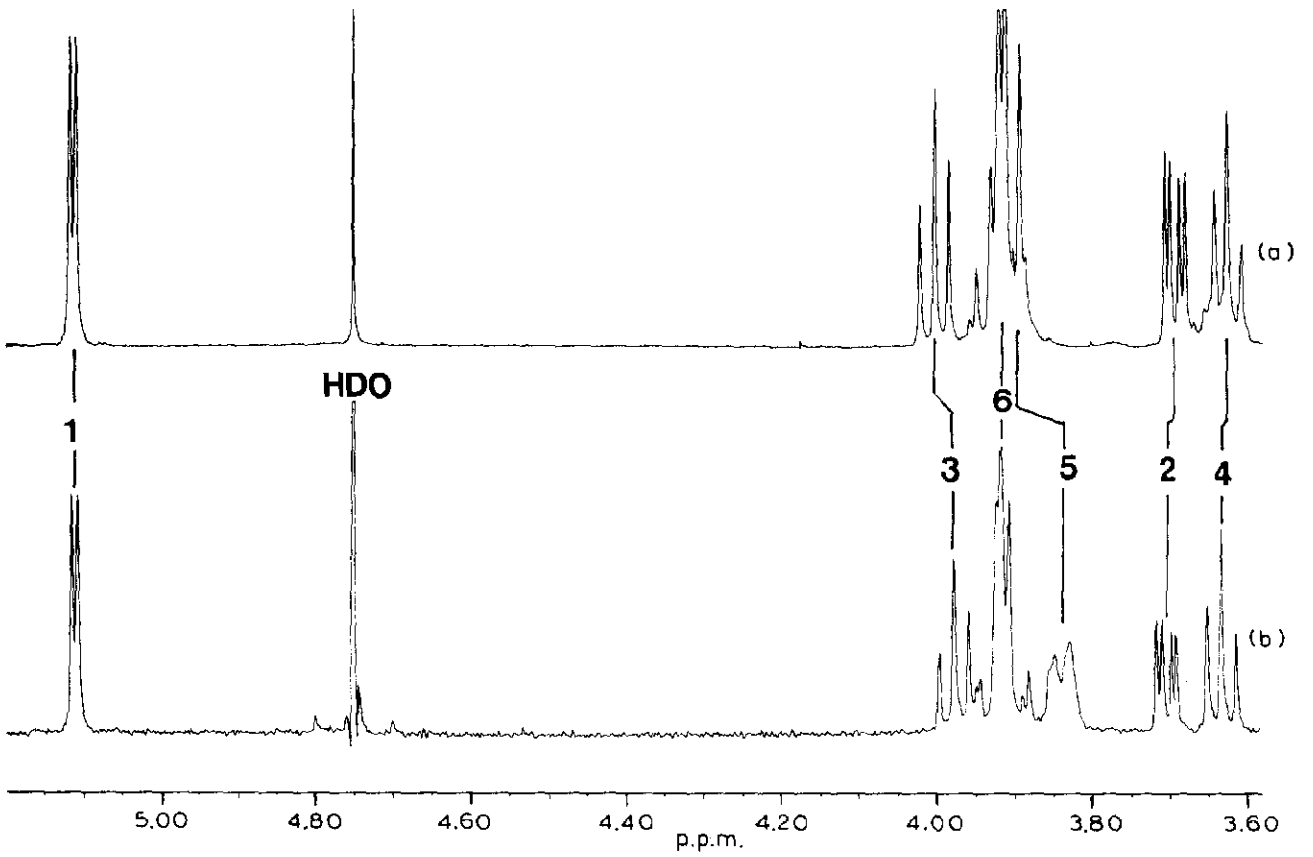

Fig. 7. ^{1}H-N.m.r. spectra (500 MHz) of (a) βCD and (b) SP/βCD in D_2O at 30° (peaks labelled 1–6 refer to hydrogen atoms of βCD).

of all ^{1}H and ^{13}C signals for SP required extensive use of 2D techniques which will be published elsewhere. Integration of the spectrum in Fig. 8b indicates clearly the 2:1 stoichiometry for the βCD/SP inclusion complex obtained in aqueous media.

Comparison of the n.m.r. spectra in Fig. 8 reveals dramatic differences in the signals from the SP. These differences are not due to complexation effects since SP

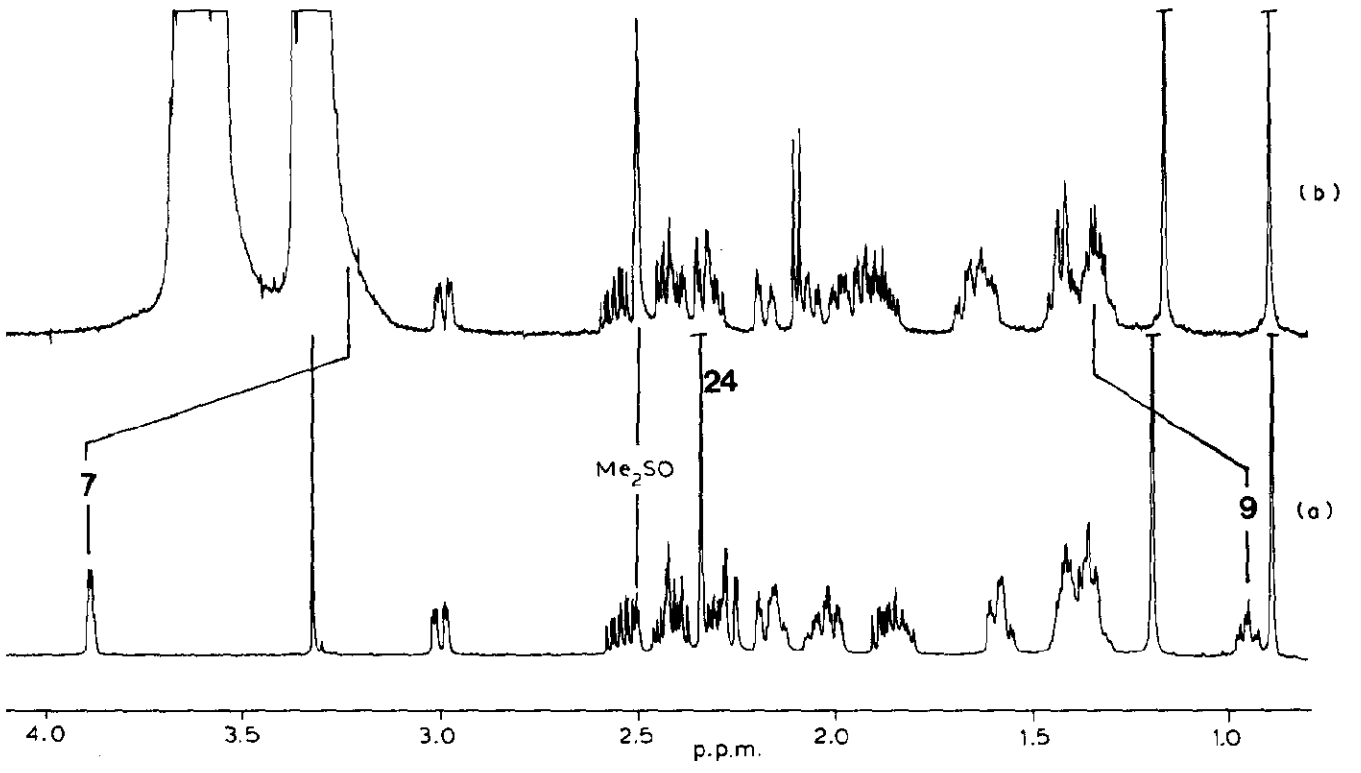

Fig. 8. ^{1}H-N.m.r. spectra (500 MHz) of (a) SP and (b) SP/βCD in $(CD_3)_2SO$ at 24°: the multiplet at 2.50 p.p.m. is from residual protons of solvent (peaks labelled 7, 9, and 24 correspond to the labelling scheme of SP).

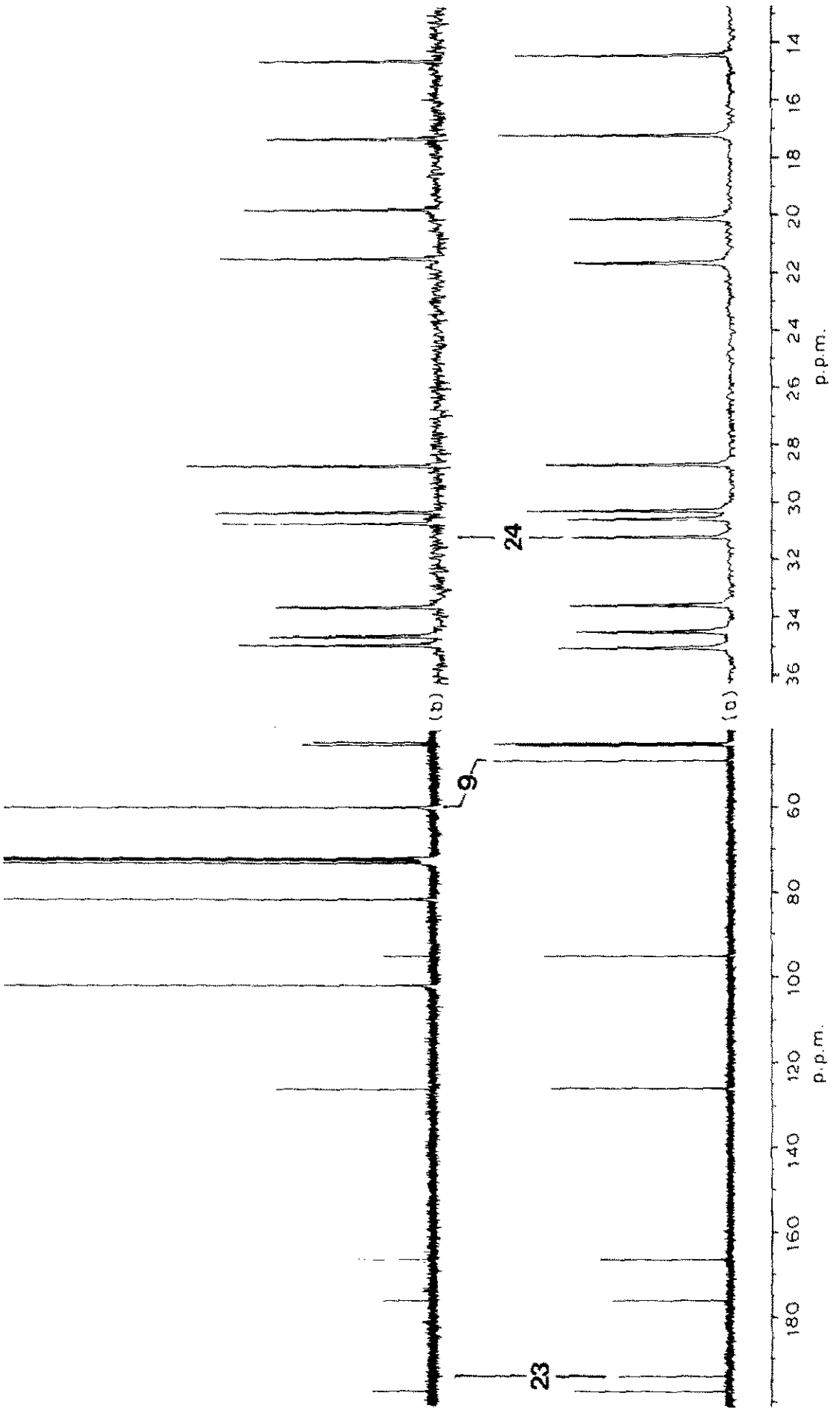

Fig. 9. ^{13}C-N.m.r. spectra (75.47 MHz, proton decoupled) of (a) SP and (b) SP/βCD at 27° in $(CD_3)_2SO$.

should not be included in the cavity of βCD in $(CD_3)_2SO$, as noted above. The most striking differences are the complete disappearance in Fig. 8b of the sharp singlet at 2.35 p.p.m. and the apparent shift of the multiplets originally at 3.88 and 0.95 p.p.m. These signals, assigned by 2D correlation experiments (COSY and Relayed COSY), correspond to the SAc group (C-24 in Fig. 2a), H-7, and H-9, respectively. COSY experiments performed on the inclusion complex proved that the last two signals were shifted to 3.3 and 1.35 p.p.m., respectively, as indicated in Fig. 8. Confirmation of the loss of the SAc group was provided by ^{13}C-n.m.r. spectroscopy. Fig. 9 shows the proton-decoupled ^{13}C-n.m.r. spectra of SP and the dissociated complex in Me_2SO. Two resonances originally present at 31.2 and 194.1 p.p.m. in the spectrum of SP are absent from that of the inclusion complex. They were previously assigned to C-24 and C-23, respectively, in Fig. 2a (*i.e.*, to the *S*-acetyl group), using direct and long-range carbon/proton correlation experiments. The resonances of C-9 (49.1 p.p.m.) and C-7 (45.1 p.p.m.) were shifted to 59.6 and 43.8 p.p.m. in the inclusion complex, and must be related to the shifts observed on the proton spectra for H-7 and H-9.

These observations confirm the absence of the *S*-acetyl group in the inclusion complex. The large shifts of the resonances of C-7, C-9, H-7, and H-9 are not related to the chemical modification but to the carbonyl group of the thioester. That the included molecule was *S*-deacetylated SP was further supported by the fact that both the ^1H- and ^{13}C-n.m.r. spectra of the inclusion complex slowly changed with time, with new lines appearing in each spectra. This effect was due to slow oxidation of the thiol group to disulphide. This inference was supported by the observation that the rate was increased markedly by bubbling dry air or oxygen through the solution. No such oxidation effect was observed in freshly prepared solutions of the complex, indicating that the thiol group is protected by the CD cage in the solid state. In the absence of βCD, no *S*-deacetylation of SP occurred.

The energy-minimized structure displayed in Fig. 4 shows the ester group to be in the contact region of the two secondary hydroxyl faces of the βCD, and this may be the cause of the *S*-deacetylation. Further interpretation must await a systematic study of the reactivity of other thioesters.

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