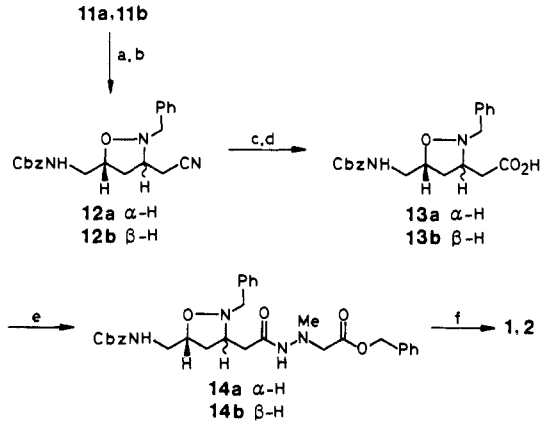
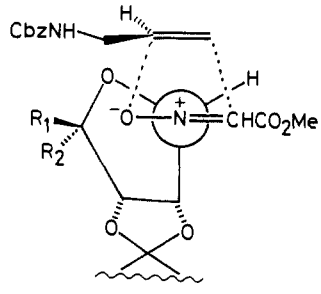
Scheme III[a]



[a] (a) TsCl, $(i\text{-Pr})_2NEt$, Et_3N/CH_2Cl_2 (1:1), 0 °C → room temperature, 10 h; (b) NaCN, $(Me)_2SO$, room temperature (2 h) → 50 °C (10 h); (c) HCl/EtOH, 0 °C → room temperature, 12 h; (d) 4% aqueous NaOH/MeOH (1:2), room temperature, 3 h; (e) $ClCO_2$ Et, Et_3N, toluene, 0 °C, 25 min, then benzyl (1-methylhydrazino)acetate, 0 °C (2 h) → room temperature (10 h); (f) H_2, Pd/C, 10% aqueous AcOH/MeOH (1:2), 3 atm, 12 h.

in 93.7% ee and 94.2% ee (determined as the (+)-MTPA esters[8]), respectively.

Both trans (**10a**) and cis (**10b**) products obtained in this cycloaddition using the *nonconjugated olefin*[9] as the dipolarophile must arise (applying Diels-Alder terminology) from the exo transition state;[10] the *E* isomer of the nitrone **8** yields the trans adduct **10a**, while the *Z* isomer yields the cis adduct **10b**. The facial selectivity observed in this cycloaddition with the *E* and *Z* nitrones may be interpreted in terms of "O-endo" transition-state model[6a,11] as shown in A, wherein, by analogy to recent reports,[12-14]



A

the electron-donating group (secondary alkyl) rather than the polar group (alkoxy) is perpendicular to the plane of the nitrogen-carbon double bond to permit the maximum orbital overlap of the participating centers, leading to the favored *re* face approach at the prochiral olefin. A similar approach to a prochiral diene has been observed in pericyclic cyclocondensation reactions of chiral sugars.[15]

Compound **11a** was converted to (+)-negamycin in six steps (Scheme III). Tosylation of **11a** followed by substitution (NaCN, Me_2SO) gave the nitrile **12a**, $[\alpha]^{17}_D$ +31.4° ($CHCl_3$), in 72% overall yield. Compound **12a** was converted to the carboxylic acid **13a**, $[\alpha]^{14}_D$ +31.7° ($CHCl_3$), in 79% yield via ethanolysis and subsequent saponification. Condensation of **13a** with benzyl (1-methylhydrazino)acetate was carried out using the mixed carboxylic acid anhydride method ($ClCO_2Et$, Et_3N)[16] affording the hydrazide **14a**, $[\alpha]^{16}_D$ +20.4° ($CHCl_3$), in 67% yield. Hydrogenolysis resulted in combined debenzylation and N-O bond cleavage; purification of the crude product by silica gel chromatography[17] gave (+)-negamycin (**1**), mp 108-115 °C dec (lit.[5] mp 110-120 °C dec), $[\alpha]^{20}_D$ +2.3° (*c* 4.07, H_2O) (lit.[5] $[\alpha]^{29}_D$ +2.5° (*c* 2, H_2O), in 75% yield. This material was found to be identical with natural negamycin (TLC, ^1H NMR, and antibacterial activity[18]).

We then completed the synthesis of optically active 3-epinegamycin (**2**) by transformation of the 3*S*,5*R*-cis isomer **11b** (Scheme III). Compound **11b** was converted in four steps to the carboxylic acid **13b**, $[\alpha]^{20}_D$ +26.0° ($CHCl_3$), which was then worked up in a manner similar to that described for **13a**, giving rise to the hydrazide **14b**, $[\alpha]^{20}_D$ +17.4° ($CHCl_3$), in 34.4% overall yield from **11b**. Hydrogenolysis of **14b** followed by silica gel chromatography[17] afforded (-)-3-epinegamycin (**2**) in 65% yield, $[\alpha]^{20}_D$ -3.2° (*c* 4.42, H_2O), mp 165-195 °C dec (for (±)-**2** lit.[3c] mp 150-180 °C dec), which had an identical ^1H NMR spectrum in a D_2O solution with that of (±)-**2**. Antibacterial activity for synthetic (-)-**2** is under investigation.

Acknowledgment. We are indebted to Professor M. Ohno of Tokyo University for kindly providing a sample of natural negamycin and also to Dr. P. Siret of I.C.I.-Pharma for a spectrum of racemic epinegamycin. We also thank Professor M. Kono and Dr. K. O'hara of Tokyo College of Pharmacy for cooperating in antibacterial tests.

(8) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(9) The nitrone cycloadditions with monosubstituted electron-rich dipolarophiles, incapable of secondary orbital interactions, proceed through exo transition states and are described as dipole LUMO controlled, resulting in 5-substituted isoxazolidines (cf.: Tufariello, J. J. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley-Interscience: New York, 1984; Vol. 2, Chapter 9).
(10) Endo transition states would greately be restricted by suffering from unfavorble steric interactions between the CH_2NHCbz group in the incoming dipolarophile **9** and the furan ring oxygen atom of the nitrone **8**.
(11) "O-Exo" transition states should be disfavored due to serious nonbonded interactions between the furan ring oxygen atom and the $CHCO_2Me$ group.
(12) DeShong, P.; Leginus, J. M. *J. Am. Chem. Soc.* **1983**, *105*, 1686.
(13) Jäger, V.; Schohe, R.; Paulus, E. F. *Tetrahedron Lett.* **1983**, *24*, 5501.
(14) Houk, K. N.; Susan, R. M.; Wu, Y.-D.; Rondan, N. G.; Jäger, V.; Schohe, R.; Fronczek, F. R. *J. Am. Chem. Soc.* **1984**, *106*, 3880.
(15) Danishefsky, S. J.; Marring, C. J.; Barbachy, M. R.; Segmuller, B. E. *J. Org. Chem.* **1984**, *49*, 4565.
(16) Vaughan, J. R., Jr.; Osato, R. L. *J. Am. Chem. Soc.* **1952**, *74*, 676.
(17) Elution was initiated with $CHCl_3$/MeOH (4:1), containing 0.5% of concentrated NH_4OH, continued with gradient solvent system and finally conducted with MeOH including 1% concentrated NH_4OH.
(18) Kono, M.; O'hara, K.; Ohmiya, K.; Iida, H.; Kibayashi, C.; Kasahara, K. *Jpn. J. Antibiot.* **1986**, *39*, 247.

On the Characterization of Intermediates in the Mitomycin Activation Cascade: A Practical Synthesis of an Aziridinomitosene

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Received March 4, 1986

Mitomycin C (mutamycin) is already a significant resource in cancer chemotherapy.[1,2] Potential second generation mitomycins are in various stages of preclinical development. It has long been recognized that mitomycins (**1**) are not per se biologically potent but require reductive priming.[3] One mode of action of suitably primed mitomycins involves the alkylation and cross-linking of DNA.[4] Furthermore, the reductive process seems to generate

(1) Carter, S. K.; Crooke, S. T. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979.
(2) (a) Cassady, J. M.; Duorose, J. D. *Anticancer Agents Based On Natural Product Models*; Academic Press: New York, 1980. (b) Remers, W. A. *Antineoplastic Agents*; Wiley: New York, 1980.
(3) (a) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 355. (b) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55. (c) Nagata, C.; Matsuyama, A. *Prog. Antimicrob. Anticancer Chemother. Proc. Int. Congr. Chemother., 6th, 1969* **1970**, *2*, 423.

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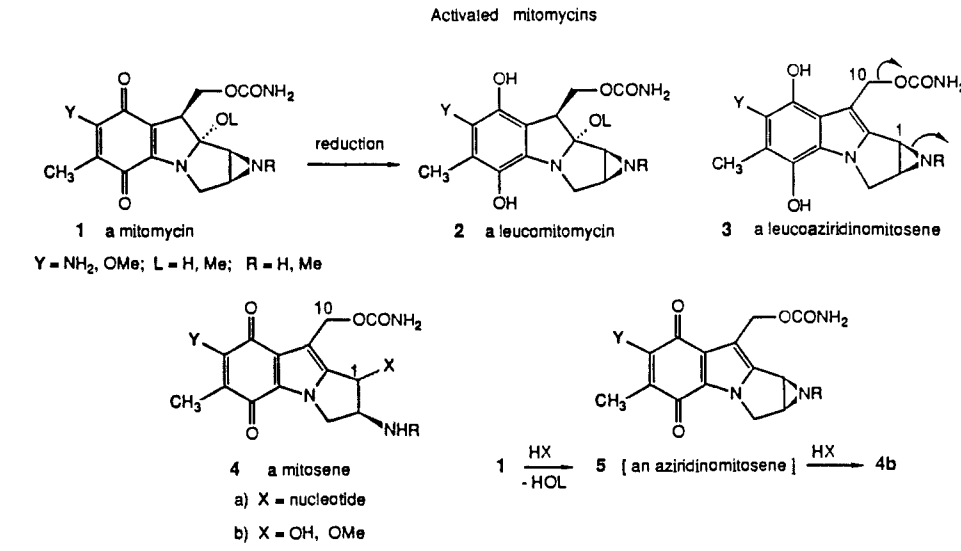
Scheme I



various agents (dioxygen or hydrogen peroxide or superoxide) that are believed to cause oxidative damage to nucleic acids.[5]

Reduction of a mitomycin **1** would afford leucomitomycin **2**, which could undergo elimination of HOL. The leucoaziridinomitosene **3** thus unveiled would be expected to be a powerful bioelectrophile.[6] The indolic system would favor cationoid character at C_1 or C_{10}, thereby facilitating attack of various DNA-centered nucleophiles. Indeed, attachment of certain sites of nucleic acids to C_1 has been demonstrated.[7] Isolated from such processes are not the immediate adducts of **3** + DNA but rather their oxidized form **4a**.

System **4a** is an example of a broader class of compounds called mitosenes. A particularly interesting type of mitosene is one which still retains the 1,2-imino linkage, i.e., an aziridinomitosene (cf. **5**). Mitosenes **4b**, but not aziridinomitosenes, have previously been obtained by acid-catalyzed elmination "LOH" from mitomycins.[8] Under these conditions, the aziridine linkage of the initially formed **5** suffers subsequent transformation to solvolysis product **4b** (Scheme I). The conversion of a mitomycin to an aziridinomitosene **5** has been accomplished in very modest yield under nearly neutral conditions by a reduction–oxidation sequence.[9] The perception which dominated the field was that the reduced leucomitomycin 2 underwent nearly spontaneous aromatization to afford the leucoaziridinomitosene **3**, which upon subsequent oxidation gave rise to **5**.[8]

Recently in our laboratory, leucomitomycins **2** were generated and characterized for the first time by reduction (H_2Pd/C–pyridine) of mitomycins. Under these conditions[10] they showed no significant tendency to suffer aromatization to **3**. Indeed, oxidation (O_2) of solutions of **2** in pyridine over Pd/C afforded high yields of mitomycin **1**, with no sign of aziridinomitosene **5**. Spectral analysis (250-MHz NMR) established that both systems **1** and **2** are stable for at least 24 h in pyridine solution.

In this paper we describe an efficient preparation of an aziridinomitosene (see compound **10**). The preparative procedure followed some new mechanistic insights into the process by which HOL is eliminated en route to mitosenes. It was then possible to prepare, for the first time, a type **3** system (see compound **11**).

Catalytic reduction of **6** (2 mL of pyridine-d_5, 105 mg of **6**,[11] 4 mg of 10% Pd–C, hydrogen bubbled through until complete decolorization) afforded a solution of leuco compound **7** (NMR analysis).[12a] Oxygenation of this solution over the catalyst resulted in immediate and quantitative conversion to **6**. A 1:1 solution of **6** and **7** was prepared. *Evaporation of this solution to dryness in the absence of oxygen followed by redissolution in pyridine afforded a 1:1 mixture of 7 and 10.* Control experiments with suitable precautions for exclusion of oxygen showed that solutions of either **6** or **7** individually do not undergo elimination upon rotary evaporation of the pyridine, followed by dissolution. Evaporation and redissolution of a 1:1 solution of **6** and **7** in the presence of external oxygen produced largely mitosene **10**[12b] with only small amounts of **6** and **7**.

It seemed that the active species undergoing elimination might be a semiquinonoidal form between the oxidation levels **6** and **7**.[13,14a]

(4) Tomasz, M.; Lipman, R.; Verdine, G. L.; Nakanishi, K. *J. Am. Chem. Soc.* **1985**, *107*, 6120.

(5) (a) Lown, J. W. *Mitomycin C: Current Status and New Developments*; Academic Press: New York, 1979. (b) Lown, J. W. *Acc. Chem. Res.* **1982**, *15*, 381. (c) Lown, J. W. *Mol. Cell. Biochem.* **1983**, *55*, 17.

(6) (a) Moore, H. W. *Science (Washington, D.C.)* **1977**, *197*, 527. (b) Moore, H. W.; Czernick *Med. Res. Rev.* **1981**, *1*, 249.

(7) For attachment of nucleic acid nucleophiles to C_1 of mitosene, see ref 4 and citations therein. Attachment of C_{10} has not been chemically demonstrated. For a recent demonstration of chemistry at C_{10}, see: Zein, N.; Kohn, H. *J. Am. Chem. Soc.* **1986**, *108*, 296.

(8) (a) Stevens, C. L.; Taylor, K. G.; Muak, M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Shah, L. G.; Uzu, K. *J. Med. Chem.* **1964**, *8* (1) 1. (b) Franck, R. W. In *Fortschr. Chem. Org. Naturst.* **1979**, *38*. (c) Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley: New York, 1978; Vol 1.

(9) (a) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W.; Webb, J. S. *J. Am. Chem. Soc.* **1964**, *86*, 1889. (b) A similar result was realized by Professor W. A. Remers who provided us with a report of previous syntheses of mitosenes and their biological evaluation.

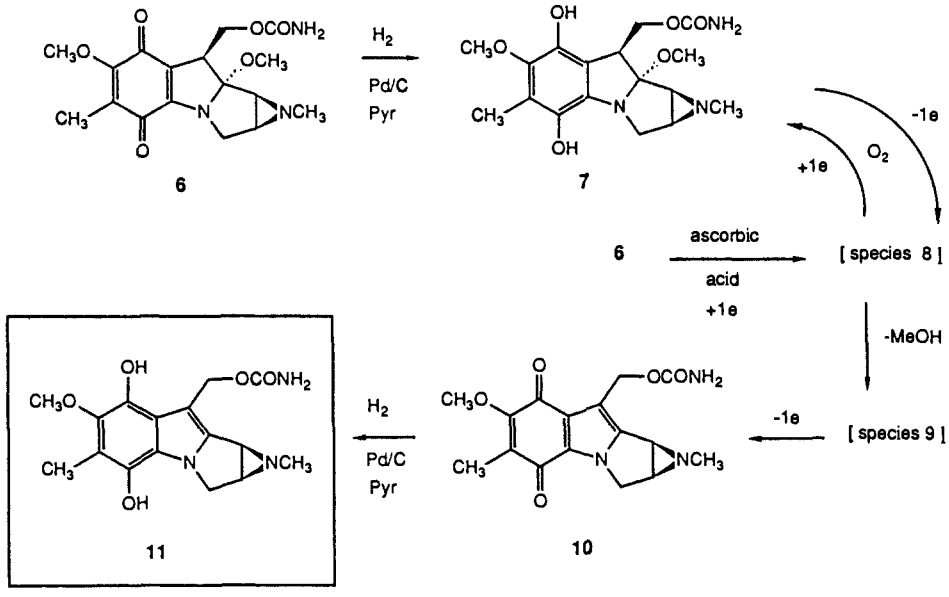
(10) Danishefsky, S.; Ciufolini, M. *J. Am. Chem. Soc.* **1984**, *106*, 6424.

(11) Compound **6** (*N*-methylmitomycin A) was prepared from mitomycin C by the method of Remers: Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767. This compound was selected for ease of handling and for spectroscopic convenience. It should be noted that Remers[9b] has reported that the aziridinomitosene derived from **6** (i.e., compound **10**) is, in fact, very active in antitumor screens.

(12) (a) Leucomitomycin **7**: IR (pyridine-d_5) 3500–3100, 1723, 1596 cm^{-1}; ^1H NMR (250 MHz, pyridine-d_5) 10.4 (br s, 1 H), 9.46 (br s, 1 H), 7.4–7.6 (br s, 2 H), 5.68 (dd, J = 10.3, 4.4 Hz, 1 H), 5.04 (dd, J = 10.3, 11.7 Hz, 1 H), 4.84 (s, H_2O), 4.18 (d, J = 11.7 Hz, 1 H), 4.18 (dd, J = 11.3, 4.5 Hz, 1 H), 3.54 (dd, J = 11.7, 2.2 Hz, 1 H), 3.47 (s, 3 H), 3.13 (s, 3 H), 2.49 (d, J = 4.5 Hz, 1 H), 2.32 (s, 3 H), 2.08 (s, 3 H), 1.96 (dd, J = 4.5, 2.2 Hz, 1 H); $[\alpha]^{25}D$ +18.6° (c 0.75, pyridine-d_5). (b) Mitosene **10**: IR ($CDCl_3$) 3526, 3426, 2923, 1727, 1658, 1641, 1582 cm^{-1}; ^1H NMR (250 MHz, pyridine-d_5) 7.7–7.6 (br s, 2 H), 5.55 (2 d, J = 13.3 Hz, 2 H), 4.8 (br s, H_2O), 4.15 (d, J = 13.8 Hz, 1 H), 3.90 (dd, buried, J = 3.9 Hz, 1 H), 3.84 (s, 3 H), 3.10 (d, J = 5.0 Hz, 1 H), 2.56 (dd, J = 3.9 Hz, 1 H), 2.1 (s, 3 H), 1.81 (s, 3 H); $[\alpha]^{25}D$ +50.4° (c = 0.58, Me_2SO), mass spectrum, m/e = 331; bright orange crystals decompose without melting at temperatures between 200 and 230 °C. (c) Leucoaziridinomitosene **11**: ^1H NMR (250 MHz, pyridine-d_5) 10.2 (br s, 1 H), 9.7 (br s, 1 H), 7.29 (br s, 2 H), 5.99 (s, 2 H), 4.5–4.8 (br s, H_2O), 4.53 (d, J = 12.0 Hz, 1 H), 4.15 (dd, J = 12.0, 3.8 Hz, 1 H), 3.63 (s, 3 H), 3.16 (d, J = 5.2 Hz, 1 H), 2.52 (dd, J = 5.0, 3.8 Hz, 1 H), 2.44 (s, 3 H), 2.1 (s, 3 H). The spectrum also contains small signals arising from a transformation of compound **11**. This will be described in due course.

(13) The possible involvement of semiquinones in the mitomycin activation cycle has been previously considered (cf. inter alia ref 3c, 5, as well as: Rao, G. M.; Lown, J. W.; Plambeck, J. *J. Electrochem. Soc.* **1977**, *124*, 195. Rao, G. M.; Begleiter, A.; Lown, J. W.; Plambeck, J. A. *Ibid.* **1977**, *124*, 199.

Scheme II



(i.e., species **8** of undefined protonation state). Elimination of methanol from **8** would afford the semiquinonoidal form of the mitosene (species **9**, of undefined protonation state). In the presence of oxygen, semiquinone **9** would be oxidized to the observed **10**. In the absence of external oxygen, **9** reacts with **8** in a disproportionation reaction to provide the observed **7** + **10**. The intervention of semiquinones in the mitomycin activation cycle is also suggested by the interesting findings of Bachur.[14b]

Support for this permissable though not obligatory formulation was obtained from the reaction of **6** in pyridine with ascorbic acid (catalytic) in pyridine. *A 90% yield of* **10** *was obtained.* A control experiment revealed that ascorbic acid in pyridine failed to effect elimination of methanol from leucomitomycin **7**. Since compound **7** is much more labile toward direct elimination than is **6**, it seems particularly unlikely that the conversion of **6** to **10** by ascorbic acid is a simple acid-catalyzed elimination process. More likely is the possibility that **6** undergoes one-electron reduction by the ascorbate,[15] producing the indolinosemiquinone equivalent **8**. This suffers elimination of methanol to afford the indolosemiquinone **9**.[16] The latter is oxidized to give **10**.

With preparatively sound routes to aziridinomitosene **10** in hand, attention was directed toward its reduction under substantially the same conditions used for the reduction of **6**. *There was thus obtained for the first time a solution of the unstable leucoaziridinomitosene* **11**. The structure of this substance follows unambiguously from its 250-MHz NMR spectrum[12c] and from its reoxidation (via oxygen) to **10** in 66% yield (Scheme II).

We are currently sorting out the chemistry of compound **11**. It is already clear that this leuco system is highly reactive. Compound **11** may be the operative bioelectrophilic form of a mitomycin. However, there is, in principle, a relationship between the methanol expulsion and the aziridine displacement processes in their dependency on electronic accession from the indolic nitrogen. Since the elimination process occurs most readily, at least in our experiment, on an oxidized (one electron) version of **7** (cf. semiquinone **8**), the aziridine opening reaction might well operate on an oxidized (one electron) version of **11** (cf. species **9**).[16]

Future research in the mitomycin area will strive for greater definition of species **8** and **9** and for elucidation of the chemistry of compound **11**, including its behavior in simulated biological systems. With the series **6** → **7** → **10** → **11** now characterized for the first time, the prospectus for progress toward these goals would seem to be realistic. Conceivably, new chemical insights could find application to the preparation of more active mitomycin congeners.

Acknowledgment. This work was supported by PHS Grant CA 28824. NMR spectra were obtained through the auspices of the Northeast Regional NSF/NMR Facility at Yale University, which was supported by NSF Chemistry Division Grant CHE 7916210. We also thank Dr. Terry Doyle of the Bristol Pharmaceutical Co. for supplying us with mitomycin C. We also acknowledge a valuable discussion of this work with Professor William Remers.

Supplementary Material Available: Copies of the NMR spectra of compounds **6**, **7**, **10**, and **11** (4 pages). Ordering information is given on any current masthead page.

(14) (a) For the involvement of semiquinones in biological electron transport processes, see: Crofts, A. R. In *The Enzymes of Biological Membranes*; Plenum Press: New York, 1985; Vol. 4, pp 347-382. (b) For a previous suggestion as to the involvement of semiquinones in the activation cascade, see: Pan, S. S.; Andrews, P. A.; Glover, C. J.; Bachur, N. R. *J. Biol. Chem.* **1984**, *259*, 959.

(15) Sieb, P. A.; Tolbert, B. M.; Bielski, B. H. J. *Ascorbic Acid: Chemistry, Metabolism and Uses*; Advances in Chemistry 200; American Chemistry Society: Washington, DC, 1982; pp 81-100.

(16) Without any justification or comment, a species analogous to **9** appears in ref 3c as a possible biological alkylating agent.

On the Interaction of Poly(pyridine)ruthenium(II) Optical Antipodes Intercalated in Montmorillonite Clay

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Received February 18, 1986

We report here the UV-vis absorption spectra of (−)-RuL_3^{2+} (L = 2,2′-bipyridine; 1,10-phenanthroline), (+)-RuL_3^{2+}, and (±)-RuL_3^{2+} adsorbed on montmorillonite clay. Our results provide compelling evidence of selective interactions between the optical antipodes of RuL_3^{2+}, when as few as 2–4% of the available cation exchange sites in clay are loaded with the Ru(II) complex.

Several unusual features of smectite clay–ML_3^{2+} adducts have been reported in recent years.[1-5] Most notable among these is

(1) Yamagishi, A.; Soma, M. *J. Am. Chem. Soc.* **1981**, *103*, 4640.
(2) Ghosh, P. K.; Bard, A. J. *J. Phys. Chem.* **1984**, *88*, 5519.
(3) DellaGuardia, R. A.; Thomas, J. K. *J. Phys. Chem.* **1983**, *87*, 990.

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