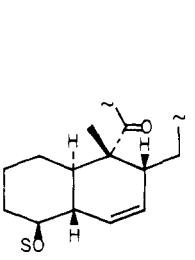
carbonyl function at C-9. It is interesting to note that closely related mechanisms can account for the formation of the octalin ring systems in chlorothricin (**5**),[20] kijanimicin,[21] and tetrocarcin,[22]



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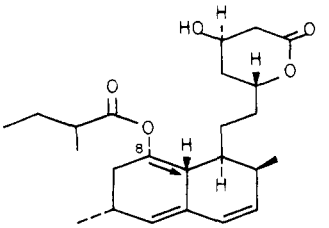

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all of which exhibit the identical cis relationship between the H-4 and H-7 hydrogen atoms (nargenicin numbering) and retain a carbonyl group adjacent to the presumptive dienophile.[23]

**Fermentation of *Nocardia argentinensis* and Incorporation of Labeled Precursors.**[10] An inoculum of *Nocardia argentinensis* Huang, ATCC 31306, was prepared by transfer of cells from a slant culture to a 500-mL Delong flask containing 70 mL of vegetative medium consisting of 10.0 g of glucose, 20.0 g of starch, 5.0 g of yeast extract, 5.0 g of enzymatic digest of casein, 0.5 g of dipotassium hydrogen phosphate, 5.0 g of meat meal, 0.002 g of $CoCl_2$, and 4.0 g of $CaCO_3$ per L of distilled water, final pH 7.1–7.2. The vegetative culture was incubated at 250 rpm and 28 °C for 4 days before being used to inoculate (3% v/v) a series of 500-mL Delong flask each containing 70 mL of a fermentation medium composed of 1.0 g of glucose, 2.5 g of enzymatic digest of casein, 5.0 g of soluble starch, 5.0 mL of corn steep liquor, 3.0 g of $CaCO_3$, and 0.002 g of $CoCl_2$ per L of distilled water, final pH 6.9–7.0. The cultures were incubated at 30 °C and 250 rpm for 4 days before extraction and isolation of antibiotic.

For feeding experiments, labeled precursors were dissolved in distilled water and added in three portions (40%, 30%, and 30%) through a disposable sterile filtration unit to each fermentation flask after 24, 48, and 72 h, respectively, during the normal fermentation period. The total dose of sodium acetate and propionate was 3.0 g/L in each case. The 90% $^{13}C$-enriched acetate and propionate precursors were diluted prior to feeding with two and five parts, respectively, of unlabeled precursor, in order to avoid excess intramolecular multiple labeling in the isolated metabolites.

After 4 days, the mycelia and fermentation broth were separated by centrifugation for 15 min at 12000*g* and each was extracted with several portions of chloroform. Concentration of the combined organic extracts gave an orange residue, which was washed and filtered through a Celite pad with 300–500 mL of ethyl acetate. After evaporation of the ethyl acetate, the residue was subjected to purification by preparative TLC (ethyl acetate–cyclohexane, 1:1; nargenicin, $R_f$ 0.27; isonargenicin, $R_f$ 0.36). The recovered nargenicin was further purified by TLC (ethyl ether–benzene, 1:1). Typical yields were 20–30 mg/L of nargenicin and 5–7 mg/L of isonargenicin.

**Acknowledgment.** This work was supported by a grant from the NIH, GM 22172. The strain of *N. argentinensis* as well as an authentic sample of nargenicin $A_1$ were kindly provided by Dr. Walter D. Celmer and Paul Watts of Ch. Pfizer, Inc., who also furnished helpful information on fermentation and isolation conditions. The Bruker WM 250 NMR used in this work was purchased with funds provided by the NSF and the Montedison Group of Milan.

**Registry No. 2**, 70695-02-2; **3**, 74666-93-6; acetic acid, 64-19-7; propionic acid, 79-09-4; carbon, 7440-44-0; oxygen, 7782-44-7.

(19) Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707.

(20) Structure: Muntwyler, R.; Keller-Schierlein, W. *Helv. Chim. Acta* **1972**, *55*, 2071. Biosynthesis: Holzbach, R.; Pape, H.; Hook, D.; Kreutzer, E. F.; Chang, C.-J.; Floss, H. G. *Biochemistry* **1978**, *17*, 556. Floss, H. G.; Chang, C.-J.; Mascaretti, O.; Shimada, K. *Planta Med.* **1978**, *34*, 345. Mascaretti, O.; Chang, C.-J.; Floss, H. G. *J. Nat. Prod.* **1979**, *42*, 455.

(21) Structure: Mallams, A. K.; Puar, M. S.; Rossman, R. R. *J. Am. Chem. Soc.* **1981**, *103*, 3940. Mallams, A. K.; Puar, M. S.; Rossman, R. R.; McPhail, A. T.; Macfarlane, R. D.; Stephens, R. L. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1497.

(22) Structure: Tomita, F.; Tamaoki, T.; Shirahata, K.; Kasai, M.; Morimoto, M.; Ohkubo, S.; Mineura, K.; Ishii, S. *J. Antibiot.* **1980**, *33*, 668. Hirayama, N.; Kasai, M.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Tetrahedron Lett.* **1980**, *21*, 2559. Biosynthesis: Tamaoki, T.; Tomita, F. *Ibid.* **1983**, *36*, 595.

(23) The fungal metabolite mevinolin (**6**), whose biosynthesis has recently been studied by Vederas (Chan, J. K.; Moore, R. N.; Nakashima, T. T.; Vederas, J. C. *J. Am. Chem. Soc.* **1983**, *105*, 3334), presents a more subtle mechanistic problem. The presence of an additional conjugated double bond within the hexalin ring system masks the original stereochemistry at the bridgehead, while the fact that the carbinyl hydrogen atom at C-8 (corresponds to C-11 of nargenicin) is derived from an acetate methyl hydrogen rules out the intermediacy of a carbonyl function at this site.

## Biosynthesis of Nargenicin and Nodusmicin

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*Received September 21, 1983*

The antibiotics nodusmicin (**1**) and nargenicin (**2**) represent a novel group of macrolide antibiotics recently isolated from fermentations of *Saccharopolyspora hirsuta* strain 367 (NRRL 12045)[1] and *Nocardia argentinensis* Huang (ATCC 31306),[2] respectively. Related compounds belonging to this reduced polyketide class have since been reported, along with additional strains producing the antibiotics.[3,4] Significant activity against anaerobes and resistant strains of *Staphylococci*, coupled with low toxicity and substantial oral activity, has led to an extensive analogue program at major pharmaceutical laboratories.[5-7]

The biosynthetic origin of the family is of particular interest due to the antibiotics' octahydronaphthalene ring system, as well as to their commercial potential. In principle, the macrolide ring could be derived from the typical *Actinomycete* acetate–propionate pathway,[8] although the acetate–methionine pathway of fungi[9] is also plausible. The oxygens at C-11, C-13, and C-17 are appropriately placed for either pattern. The C-2 oxygen could indicate a glycolate origin for the C-1,C-2 unit as in geldanamycin[10] and leucomycin (via glycerol),[11] or it could be introduced at an acetate-derived carbon. The pyrrole carbonyl unit is presumably derived from glutamate via proline.[12]

In the present work, fermentation studies designed to optimize production of nargenicin from *N. argentinensis* led to the detection

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(2) Celmer, W. D.; Chmurny, G. N.; Moppett, C. E.; Ware, R. S.; Watts, P. C.; Whipple, E. B. *J. Am. Chem. Soc.* **1980**, *102*, 4203–4209.

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(5) Magerlein, B. J.; Reid, R. J. *J. Antibiot.* **1982**, *35*, 254–255.

(6) Magerlein, B. J.; Mizsak, S. A. *J. Antibiot.* **1982**, *35*, 111–112.

(7) Magerlein, B. J. "Abstracts of Papers", 182nd National Meeting of the American Chemical Society, New York, NY, Aug 23–28, 1981; American Chemical Society: Washington, DC, 1981; MEDI 72.

(8) (a) Corcoran, J. W. In "Antibiotics. Vol. IV. Biosynthesis"; Corcoran, J. W., Ed.; Springer-Verlag: Berlin, 1981; pp 132–174. (b) Ōmura, S.; Nakagawa, A. *Ibid.*; pp 175–192.

(9) Turner, W. B. "Fungal Metabolites"; Academic Press: New York, 1971; pp 74–213.

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(12) Florkin, M., Stotz, E., Eds. "Comprehensive Biochemistry"; Elsevier: Amsterdam, 1968; Vol. 20, pp 234–235.

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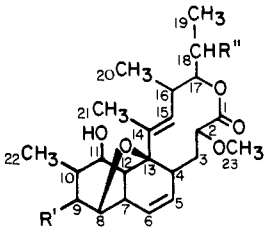
Table I. Incorporation of Labeled Precursors into Nargenicin

| precursor | | | nargenicin | | | | |
|---|---|---|---|---|---|---|---|
| compound | sp act., mCi/mmol | amount, μCi | yield, mg | sp act., μCi/mmol | diln × $10^{-3}$ | activity, μCi × $10^{3}$ | incorpn, % |
| sodium [*carboxy*-$^{14}$C]propionate | 56.3 | 1.05 | 8.7 | 1.89 | 29.8 | 31.9 | 3.04 |
| sodium [2-$^{14}$C]propionate | 52.0 | 0.593 | 3.2 | 2.74 | 19.0 | 17.0 | 2.87 |
| sodium [*methyl*-$^{14}$C]propionate | 6.3 | 0.557 | 4.8 | 2.59 | 2.43 | 24.3 | 4.36 |
| L-[*methyl*-$^{14}$C]methionine | 54.0 | 8.84 | 11.2 | 19.3 | 2.80 | 420 | 4.75 |
| D-[*U*-$^{14}$C]glucose | 255 | 16.7 | 12.8 | 0.958 | 267 | 23.8 | 0.14 |
| sodium [*carboxy*-$^{14}$C]acetate | 2.2 | 7.34 | 7.7 | 0.706 | 3.12 | 10.5 | 0.14 |
| sodium [2-$^{14}$C]malonate | 55.8 | 2.01 | 8.3 | 0.124 | 450 | 2.01 | 0.10 |
| [1,4-$^{14}C_2$]succinic acid | 8.8 | 0.141 | 5.6 | 0.0097 | 907 | 0.11 | 0.08 |
| [2,3-$^{14}C_2$]succinic acid | 16.7 | 0.724 | 8.9 | 0.256 | 65.2 | 4.43 | 0.61 |
| [1-$^{14}$C]glyceric acid | 16 × $10^{3}$ | 0.646 | 5.6 | 0.092 | ~$10^{5}$ | 0.001 | 0.01 |
| [$^{14}C_2$]oxalic acid | 4.09 | 0.335 | 2.9 | 0.007 | 584 | 0.04 | 0.01 |
| sodium [1-$^{14}$C]glycolate | 5.0 | 6.18 | 2.5 | 0.128 | 39.1 | 0.623 | 0.02 |
| [U-$^{14}$C]glutamic acid | 195 | 0.071 | 8.0 | 0.0088 | ~$10^{4}$ | 0.136 | 0.19 |
| L-[U-$^{14}$C]proline | 10.0 | 2.4 | 8.1 | 0.721 | 13.9 | 11.3 | 0.47 |

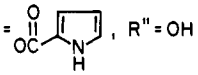
| precursor | | | nodusmicin | | | nargenicin | | |
|---|---|---|---|---|---|---|---|---|
| | enrichment, % $^{13}$C | amount, mg | yield, mg | incorpn, % | diln | yield, mg | incorpn, % | diln |
| sodium [*carboxy*-$^{13}$C]acetate[a] | 90 | 300 | 10.7 | 0.19 | 3.59 | 20.6 | 0.47 | 2.33 |
| sodium [*methyl*-$^{13}$C]acetate[b] | 90 | 235 | 6.3 | 0.44 | 11.85 | 11.8 | 0.11 | 7.43 |
| sodium [1,2-$^{13}C_2$]acetate[b] | 90 | 280 | 12.4 | 0.10 | 8.51 | 11.8 | 0.12 | 5.53 |
| sodium [*carboxy*-$^{13}$C]propionate[a] | 90 | 150 | 12.0 | 1.01 | 1.80 | 15.1 | 1.11 | 1.69 |
| L-[*methyl*-$^{13}$C]methionine[a] | 90 | 40 | 8.1 | 1.03 | 6.89 | 10.8 | 1.73 | 4.51 |

[a] Incorporations and dilutions were calculated on the basis of results obtained from a small quantity of radiolabeled precursor added. [b] Seed cultures 7 days old were used in place of the standard cultures (3 days old). Incorporations were based on a small quantity of sodium [*carboxy*-$^{14}$C]acetate added to the fermentations.

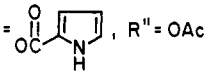
of three biologically active metabolites previously unreported from this culture. These were isolated and identified as nodusmicin (**1**), 18-*O*-acetylnodusmicin (**3**), and 18-*O*-acetylnargenicin (**4**).



**1**: R' = R" = OH

**2**: R' =  , R" = OH

**3**: R' = OH, R" = OAc

**4**: R' =  , R" = OAc

Compound **1** was identified by direct comparison with nodusmicin, while **3** and **4** were assigned structures based primarily on $^{13}$C NMR chemical shifts.[13,14] Additional resonances at 171.9 ppm for **3** and 170.4 ppm for **4** (singlets, SFORD) as well as at 21.1 ppm for both (quartets, SFORD), allowed assignment of **3** and **4** as acetates. The other major variations in the $^{13}$C spectra between nodusmicin and nargenicin standards and **3** and **4** were respective downfield shifts of 4.4 and 3.5 ppm for C-18, upfield shifts of 4.2 and 3.9 ppm for C-19, and upfield shifts of 1.5 and 2.3 ppm for C-17, while signals for C-9 and C-11 did not shift. Electron ionization mass spectrometry assigned the expected molecular weights of 464 and 557 to **3** and **4**, respectively.

The coproduction of nodusmicin and nargenicin under selected fermentation conditions allowed simultaneous biosynthetic studies. Production of the antibiotics was achieved on a complex medium to which $^{14}$C-labeled precursors were added (Table I). High incorporation of [1-$^{14}$C]-, [2-$^{14}$C]-, and [3-$^{14}$C] propionates and L-[*methyl*-$^{14}$C]methionine into nargenicin suggested a propionate origin for the aliphatic methyls and a methionine origin for the methoxyl group. None of the oxygenated units examined (glycerate, glycolate, oxalate) as potential precursors for C-1 and C-2 was incorporated. Incorporation of [*carboxy*-$^{14}$C]acetate and of [*methylene*-$^{14}$C]malonate was significant, arguing these as possible, but inefficient, $C_2$ precursors. Incorporation of [2,3-$^{14}C_2$]succinate was higher. Both [U-$^{14}$C]glutamate and L-[U-$^{14}$C]proline were incorporated.

The feeding of [*carboxy*-$^{13}$C]propionate and L-[*methyl*-$^{13}$C]-methionine confirmed the propionate origin of the methyl groups in nodusmicin and nargenicin (Table II). Consistent with the propionate pathway, C-9, C-13, C-15, and C-17 of both antibiotics were labeled by [*carboxy*-$^{13}$C]propionate, while only the methoxyl carbon was labeled by L-[*methyl*-$^{13}$C]methionine. [*carboxy*-$^{13}$C]Acetate enriched C-1, C-3, C-5, C-7, and C-11, accounting for all the subunits of the polyketide chain.

As shown in Table II, [*carboxy*-$^{13}$C]acetate enriched C-9, C-13, C-15, and C-17 to an even greater extent than the acetate-derived carbons. This indicates an active tricarboxylic acid (TCA) cycle,[15] transforming acetate to succinate, which is then converted to methylmalonate (and propionate) by an active methylmalonyl-CoA mutase.[16] This interpretation is supported by the higher

(13) The spectrum of **3** was obtained in methanol-$d_4$ due to low solubility in acetone.

(14) The $^{13}$C assignments of nodusmicin by Whaley et al.[1] and of nargenicin by Celmer et al.[2] apparently agree for all carbons except C-2 and C-8, which were reversed, and C-7, C-10, and C-12, which were interchanged. We believe the assignments in Table II to be correct. The latter three (C-7, C-10, and C-12) can be distinguished by the observation that [1-$^{13}$C]acetate enhances the signal at 39.0 ppm (C-7) and [2-$^{13}$C]acetate that near 50 ppm (C-12), leaving that at 36.3 ppm in **1** (34.8 in **2**) to C-10. While this argument involves elements of circularity, it is acceptable in the present case where acetate has already been shown to provide the $C_2$ units concerned. Furthermore, preliminary results (W.C.S.) from feeding experiments with [1,2-$^{13}$C]acetate gave the same coupling constant (61.8 Hz) for C-1 and the carbon at 83.4 ppm (C-2) of **2**. These assignments agree with those in the accompanying article by Cane and Yang.[18]

(15) Lehninger, A. L. "Principles of Biochemistry", Worth: New York, 1982; p 443.

(16) Reference 15, p 522.

Table II. $^{13}C$ Enrichment of Antibiotic Carbons by Precursors

| carbon | $\delta^{b,c}$ 1 | $\delta^{b,c}$ 2 | enrichment[a] sodium [1-$^{13}C$]-acetate 1 | 2 | sodium [2-$^{13}C$]-acetate 1 | 2 | sodium [1-$^{13}C$]-propionate 1 | 2 | L-[*methyl*-$^{13}C$]-methionine 1 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 173.5 | 172.7 | | 3.6 | 1.3 | 0.8 | | | | |
| 2 | 85.6 | 83.4 | 0.7 | 1.1 | 2.7 | 3.8 | 1.0 | 1.2 | 1.1 | 2.0 |
| 3 | 35.7 | 34.3 | 3.7 | 4.2 | 0.7 | 1.1 | 0.9 | 1.1 | 0.6 | 1.8 |
| 4 | 43.5 | 43.0 | 0.9 | 1.0 | 2.2 | 3.4 | 0.9 | 0.7 | 1.4 | 1.6 |
| 5 | 133.3 | 132.8 | 3.7 | 4.4 | 1.4 | 0.9 | 1.5 | 1.1 | 0.7 | 1.4 |
| 6 | 129.1 | 127.5 | 1.0 | 1.2 | 3.9 | 3.4 | 1.5 | 1.5 | 1.0 | 1.8 |
| 7 | 39.0 | 39.0 | 4.0 | 4.8 | 1.0 | 1.1 | 1.2 | 1.4 | 1.1 | 1.7 |
| 8 | 83.4 | 81.2 | 0.7 | 1.2 | 3.4 | 3.4 | 0.9 | 1.1 | 0.9 | 2.4 |
| 9 | 72.7 | 73.7 | 6.0 | 8.7 | 0.7 | 1.1 | 9.7 | 17.4 | 0.9 | 2.3 |
| 10 | 36.3 | 34.8 | 0.8 | 1.2 | 0.7 | 1.0 | 1.2 | 1.6 | 0.9 | 2.2 |
| 11 | 75.6 | 76.0 | 2.9 | 4.5 | 0.6 | 1.1 | 1.2 | 2.0 | 1.0 | 1.7 |
| 12 | 50.4 | 49.1 | 0.8 | 0.6 | 2.2 | 3.3 | 1.0 | 1.0 | 0.7 | 1.2 |
| 13 | 89.4 | 89.1 | 6.3 | 6.1 | 1.1 | 0.6 | 7.3 | 8.0 | | |
| 14 | 136.1 | 134.3 | | | 0.7 | 0.8 | | | | |
| 15 | 131.3 | 131.3 | 5.6 | 6.9 | 0.8 | 0.7 | 8.8 | 11.4 | 0.8 | 1.5 |
| 16 | 33.5 | 32.7 | 0.8 | 1.0 | 1.4 | 1.1 | 1.0 | 1.1 | 1.0 | 1.9 |
| 17 | 79.1 | 79.0 | 5.9 | 6.6 | 1.3 | 0.8 | 9.4 | 11.2 | 0.7 | 2.0 |
| 18 | 66.0 | 66.4 | 0.7 | 1.1 | 0.7 | 1.1 | 0.9 | 1.3 | 1.2 | 1.7 |
| 19 | 21.7 | 20.6 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 20 | 13.6 | 12.9 | 0.7 | 0.9 | 1.0 | 1.0 | 0.8 | | 0.6 | 1.5 |
| 21 | 17.5 | 17.0 | 0.4 | 0.8 | 0.6 | 0.9 | 0.5 | 0.9 | 0.7 | 1.2 |
| 22 | 16.0 | 15.5 | 0.7 | 0.8 | 0.9 | 1.0 | 0.7 | 1.0 | 1.1 | 1.3 |
| 23 | 57.5 | 57.7 | 1.0 | 1.0 | 0.6 | 0.7 | 1.3 | 0.9 | 27.2 | 21.4 |

[a] Peak height times natural abundance, based on peak height of 1.0 for C-19 as an internal standard. [b] Parts per million from $Me_4Si$; nodusmicin spectra taken in acetone, nargenicin spectra taken in $CDCl_3$. [c] Resonances of the pyrrole carbonyl group have been omitted for nargenicin. No enrichment of these carbons was observed.

level of incorporation of succinate than acetate (Table I). From the $^{14}C$ incorporation data it appears that most of the acetate incorporated is first processed through the TCA cycle, rather than being directly incorporated as malonyl-CoA. However, the extent to which acetate was converted into propionate varied considerably in four experiments employing different soil stocks. For example, in the experiment with [2-$^{13}C$]acetate in Tables I and II very little labeling of propionate by acetate was observed.

**$^{13}C$ NMR spectra** were obtained on a Varian XL 200 spectrometer using a 5-mm probe with identical operating parameters for each set of antibiotics. Samples were diluted with unenriched nargenicin or nodusmicin, as desired.

**Radiolabel Determination.** Samples of the two antibiotics were first dissolved in methanol to a final concentration of 1 mg/mL and each methanolic solution (1.0 mL) was diluted with 15 mL of Aquasol II (New England Nuclear). Disintegrations per minute (corrected for background) were determined on a Tracor Analytic BetaTrac, Model 6895, liquid scintillation counter.

**Simultaneous Production of 1, 3, and 4**: A seed medium [TY medium, consisting of Tryptone (Difco), 0.5%; yeast extract (Gibco), 0.3%; and tap water, 100 mL in a 500-mL Erlenmeyer flask] was inoculated from a dried soil stock of *Nocardia argentinensis* Huang (ATCC 31306) and incubated at 30 °C on a rotary shaker at 250 rpm. After 72 h, 5-mL aliquots were removed and inoculated into 100-mL portions of the production medium [glucose, 2.2%; Peptone (Difco), 0.5%; beef extract, 0.5%; yeast extract, 0.5%; N-Z amine type B (Sheffield), 0.3%; NaCl, 0.5%, with tap water] in 500-mL flasks. The medium was incubated for 120 h then filtered through glass wool. The clarified broth was extracted with two half-volumes of ethyl acetate and the combined organic layers were taken to dryness. After trituration with hexane, the remaining solids were dissolved in chloroform and separated on silica gel with a gradient of chloroform–methanol. Fractions were combined on the basis of TLC (Merck silica gel 60, $CHCl_3$:MeOH, 9:1, visualized by spraying with EtOH:$H_2SO_4$:*p*-anisaldehyde, 90:5:5, followed by warming).[17] The respective $R_f$ values for **1**, **2**, **3**, and **4** in this system are 0.44, 0.62, 0.66, and 0.87.

**Production of nodusmicin and nargenicin** was achieved by the same procedure as in the preceding paragraph except for the production medium (soluble starch, 2%; corn steep liquor, 2%; corn meal, 2%; $CaCO_3$, 0.3%, 100 mL in 500-mL flasks). After a 12-h incubation (30 °C, 250 rpm), precursors were added, and fermentation was continued for 132 h. The antibiotics were isolated from the filtered broth by extracting twice with quarter-volumes of ethyl acetate followed by evaporating the combined organic phases. After hexane trituration of the resulting syrups, the remaining chloroform-soluble materials were processed over Brinkmann silica gel ($CHCl_3$:MeOH 98.5:1.5 for nargenicin and $CHCl_3$:MeOH 97:3 for nodusmicin). Fractions were pooled on the basis of TLC. Yields of pure nargenicin ranged from 20 to 40 μg per mL of fermentation medium and of pure nodusmicin from 10 to 20 μg per mL.

**Acknowledgment.** This work was supported in part by a grant (AI 01278) from the National Institute of Allergy and Infectious Diseases. We thank A. Dietz and Drs. J. E. Grady and H. A. Whaley, The Upjohn Co., Kalamazoo, MI, for their advice and assistance throughout this project.

**Registry No.** **1**, 76265-48-0; **2**, 70695-02-2; **3**, 88343-90-2; **4**, 74686-27-4; propionic acid, 79-09-4; methionine, 63-68-3; acetic acid, 64-19-7; malonic acid, 141-82-2; succinic acid, 110-15-6; glutaric acid, 56-86-0; proline, 147-85-3.

(17) This reagent, a modification of that used by Calton et al. (Calton, G. J.; Ranieri, R. L.; Espenshade, M. A. *J. Antibiot.* **1978**, *31*, 38–42), was quite useful in that all compounds containing the pyrrole carbonyl unit developed as bright yellow spots while those lacking the unit became bright pink.

(18) Cane, D. E.; Yang, C.-C. *J. Am. Chem. Soc.*, preceding paper in this issue.

## A Novel Cubane-Type $Mo_4S_4$ Cluster

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The cubane-type $Fe_4S_4$[1] and $MoFe_3S_4$[2] clusters have been attracting much interest as the synthetic analogues of the active site in the ferredoxine[3] and FeMo protein of the nitrogenases.[4]

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