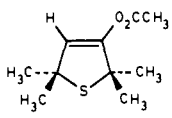
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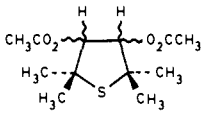
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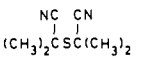
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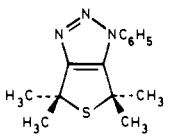
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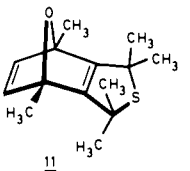
11

products were completely characterized by spectral and analytical techniques.[14] The product distributions from various experiments are given in Table I.

The enol acetate **7** can be formed by trapping of **1** by acetic acid present in the reaction medium; there is precedent for such trapping.[11] The diacetate **8** (cis, trans mixture), probably formed by reaction of acetic acid with diazo functionalities, is stable under the reaction and workup conditions and does not provide **7**. Addition of dry pyridine to the reaction mixture of experiment A (Table I) led to decreased yields of **7** and the formation of an extremely unstable product that may be an addition product of **1** and pyridine, although its structure could not be established.

Even stronger evidence for the existence of **1** is the isolation of cycloadduct **10** (experiment B, Table I) and cycloadduct **11** (experiment C, Table I). The latter cycloadduct is surprisingly stable to acid, probably because of steric protection of the oxanorbornadiene by the methyl groups. The adduct **11** is also isolated on using $MnO_2$ as the oxidant (Experiment D). The isolation of two different cycloadducts under differing reaction conditions as well as the acetic acid addition product **7** are experimental observations that are difficult to explain except in terms of a free transient species **1**.

The efficiency of formation of **1** depends greatly on the reaction conditions. For example, in experiments D and E wherein $MnO_2$ is used as oxidant, the competing pathway of cleavage of **5** perhaps through bis(diazo) compound **6** to dinitrile **9** accounts for all or nearly all the isolated product. This mode of fragmentation also competes, but less well, in other experiments.

Acetylene **1** has, to the best of our knowledge, the smallest ring of any heteroatom-containing cyclic acetylene yet reported[15] (there is evidence for the five-membered heteroaryne, 2,3-thiophyne,[3d,f] but not for 3,4-thiophyne[16]). The ease of generation of **1** suggests that with optimalization of the synthetic approach that it should be quite readily available. The relative stability of **1** is also greatly encouraging, especially the fact that there is no noticeable tendency to eliminate the sulfur bridge to form 1,1,4,4-tetramethyl-1,2,3-butatriene. The synthesis of other five-membered acetylenes structurally related to **1** should be possible.

(14) Spectral and analytical data for **7-11**. **7**: $^1$H NMR ($CDCl_3$) $\delta$ 1.48 (s, 6, $2CH_3$), 1.51 (s, 6, $2CH_3$), 2.18 (s, 3, $CH_3$), 5.63 (s, 1, vinyl H); $^{13}$C NMR ($CDCl_3$) $\delta$ 21.0 (q, $J_{C-H}$ = 132 Hz, $CH_3$), 30.6 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 33.4 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 51.3 (s, quaternary C), 56.0 (s, quaternary C), 121.3 (d, $J_{C-H}$ = 168 Hz, vinyl C), 149.8 (s, vinyl C), 168.0 (s, C=O); IR (neat) 1775 (C=O) and 1670 cm$^{-1}$ (C=C); exact mass, calcd. $m/e$ for $C_{10}H_{16}O_2S$ 200.088; found $m/e$ 200.087. **8** (isolated as cis-trans mixture), **8** (cis): $^1$H NMR ($CDCl_3$) $\delta$ 1.42 (s, 6, $2CH_3$), 1.50 (s, 6, $2CH_3$), 2.08 (s, 6, $2CH_3CO$), 5.29 (s, 2, tertiary H). **8** (trans): $^1$H NMR ($CDCl_3$) $\delta$ 1.42 (s, 12, $4CH_3$), 2.08 (s, 6, $2CH_3CO$), 5.35 (s, 2, tertiary H); IR (cis-trans mixture, neat) 1750 cm$^{-1}$; exact mass (cis-trans mixture), calcd. $m/e$ for $C_{12}H_{20}O_4S$ 260.108; found $m/e$ 260.108. **9**: $^1$H NMR ($CDCl_3$) $\delta$ 1.82 (s, $CH_3$); $^{13}$C NMR ($CDCl_3$) $\delta$ 28.2 (q, $J_{C-H}$ = 130 Hz, $CH_3$), 37.2 (s, quaternary C), 122.4 (s, C≡N); IR (neat) 2230 cm$^{-1}$ (C≡N); exact mass, calcd $m/e$ for $C_8H_{12}N_2S$ 168.072; found $m/e$ 168.074. **10**: $^1$H NMR ($CDCl_3$) $\delta$ 1.62 (s, 6, $2CH_3$), 1.87 (s, 6, $2CH_3$), 7.55 (br s, 5, aromatic); $^{13}$C NMR ($CDCl_3$) $\delta$ 31.8 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 32.1 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 47.3 (s, quaternary C), 48.1 (s, quaternary C), 125.8 (d, $J_{C-H}$ = 162 Hz, aromatic C), 129.1 (d, $J_{C-H}$ = 162 Hz, aromatic C), 129.9 (d, $J_{C-H}$ = 162 Hz, aromatic C), 136.2 (s, quaternary aromatic C), 144.0 (s, vinyl C, 158.4 (s, vinyl C); IR (KBr) 1043 and 1008 cm$^{-1}$ (triazole); exact mass, calcd. $m/e$ for $C_{14}H_{17}N_3S$ 259.114; found $m/e$ 259.112. **11**: $^1$H NMR ($CDCl_3$) $\delta$ 1.35 (s, 6, $2CH_3$), 1.63 (s, 6, $2CH_3$), 1.66 (s, 6, $2CH_3$), 6.97 (s, 2, vinyl H); $^{13}$C NMR ($CDCl_3$) $\delta$ 15.7 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 27.7 (q, $J_{C-H}$ = 128 Hz, $CH_3$), 30.7 (q, $J_{C-H}$ = 132 Hz, $CH_3$), 54.2 (s, quaternary C), 89.5 (s, quaternary C), 147.3 (d, $J_{C-H}$ = 175 Hz, vinyl C), 163.9 (s, vinyl C); IR (KBr) 1310, 1285, 1220, 1150, 1134, 877, 861, and 731 cm$^{-1}$ (not specifically assigned); exact mass, calcd. $m/e$ for $C_{14}H_{20}OS$ 236.123; found $m/e$ 236.125.

(15) Draber (Draber, W. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 72) has suggested that an imide of acetylenedicarboxylic acid is formed as a reactive intermediate. We believe that the results described in this publication can be more logically interpreted in terms of classical cycloaddition chemistry.

(16) Arynes, B. F.; Longworth, G. W.; McOmie, J. E. W. *Tetrahedron* **1975**, *31*, 1755-1760.

## One-Electron Electrochemical Reduction of a Ferrous Porphyrin Dioxygen Complex

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*Received September 2, 1980*

Despite the fact that cytochromes P-450 have been known for less than two decades,[1] considerable information concerning their modes of action as monoxygenases is known.[2] In the case of $P\text{-}450_{cam}$ the resting enzyme is a low-spin ferric hemoprotein[3] which upon binding of substrate changes to high spin in order to facilitate the first of two one-electron reductions. The first one-electron reduction generates a low-spin ferrous complex which reversibly can bind both dioxygen and carbon monoxide. The unusual optical spectrum of the CO complex allowed us,[4] and others,[5] to show that the sixth axial ligand is a thiolate anion. Moreover, it is now known that the binding of dioxygen to the ferrous heme, which is the next step in the enzymatic cycle, leaves the thiolate coordinated as the sixth axial ligand.[6,7] To this stage in the enzymatic cycle the rates and nature of the axial ligation and electronic configurations around the heme are reasonably well understood. The next step is the second one-electron reduction of the $O_2$ complex. Little is known and even less is understood about this and the subsequent steps leading to the oxygenation of substrate. The ferrous porphyrin dioxygen complex readily autoxidizes to ferric porphyrin, likely via generation of superoxide,[7] suggesting that there is some charge transfer from iron to oxygen and that this oxygenated porphyrin has some ferric superoxide character.[8] One might then envisage the additional electron from the second reduction going into an orbital in either the iron, to give formally a ferrous superoxide complex, or the dioxygen, to give formally a ferric peroxide complex.[9,10]

(1) Omura, T.; Sato, R. *J. Biol. Chem.* **1962**, *237*, 1375-1376.
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(3) Cytochromes P-450 are found in the microsomes of kidney, liver, lung, adrenal gland, and the pancreas from various mammals where the enzymes are membrane bound. In addition, *P. putida* provides a crystalline nonmembrane-bound enzyme which metabolizes camphor. While each of these enzyme systems controls different chemistry, their basic modes of action are similar.
(4) Chang, C. K.; Dolphin, D. *J. Am. Chem. Soc.* **1975**, *97*, 5948-5950.
(5) Collman, J. P.; Sorrell, T. N. *J. Am. Chem. Soc.* **1975**, *97*, 4133-4134.
(6) Dolphin, D.; James, B. R.; Welborn, H. C. *Biochem. Biophys. Res. Commun.* **1979**, *88*, 415-421.
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(9) Nordblom, G. D.; White, R. E.; Coon, J. J. *Arch. Biochem. Biophys.* **1976**, *175*, 524-533.
(10) Nordblom, G. D.; Coon, M. J. *Arch. Biochem. Biophys.* **1977**, *180*, 343-347.

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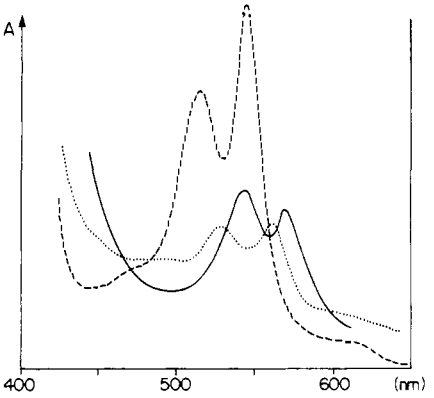



**Figure 1.** Optical spectra, in $Me_2SO/CH_3CN$ at −25 °C, of $Fe^{II}(OEP)$ (---) and $[Fe(OEP)O_2]^-$ (—) prepared by the one-electron electrochemical reduction of $Fe^{II}(OEP)L(O_2)$ (···).

We have approached this problem by examining the direct one-electron reduction of a dioxygen complex of ferrous octaethylporphyrin $[Fe^{II}(OEP)L(O_2)]$, L = solvent. Cyclic voltammetry on this complex in $CH_3CN/Me_2SO$ (1:1 v/v) at −25 °C showed a fully reversible couple at −0.24 V vs. Ag/AgCl. A "bulk" electrolysis was carried out in a 1-mm cuvette containing a thin platinum gauze electrode through which the monitoring light beam passed. Electrolysis was carried out by using a standard three-electrode system.[11] Slow diffusion at the low temperature allowed the anode to be placed a short distance from the cathode without separation by a divider. Initially a high concentration of electrolyte solution (tetra-*n*-butylammonium perchlorate ~0.3 M) containing $\sim 10^{-4}$ M ferrous OEP in $CH_3CN/Me_2SO$ was cooled to −25 °C and dioxygen added to give complete formation of $[Fe^{II}(OEP)L(O_2)]$. Argon was then bubbled to removed excess dioxygen, the spectrum being monitored carefully to ensure that no deoxygenation occurred. Reduction at −0.5 V[12] ($n = 1.0 \pm 0.1$ $e^-/Fe$) brought about the optical changes recorded in Figure 1. The oxygenated ferrous species was sufficiently stable ($t_{1/2}$ = 10 min) under these conditions to observe its reduction without spectral interference from decomposition to the $\mu$-oxo dimer.

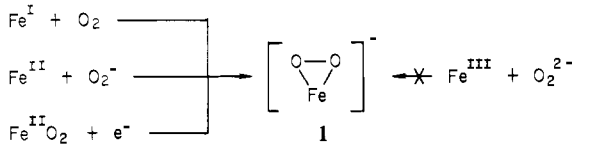
The product formed by this one-electron electrochemical reduction is identical with that produced by reacting either $Fe^{I}OEP$ with dioxygen[13] or $Fe^{II}OEP$ with superoxide.[15] In addition to the identical optical spectrum the reduced oxygenated species shows a rhombic EPR spectrum (at 77 K) with a sharp resonance at $g = 4.2$ and a weaker signal at $g = 2$. Both McCandlish et al.[16] and Reed et al.[14] observed similar EPR signals, and on the basis of these and infrared measurements, the structure of the reduced species has been formulated[16] as a high-spin ferric $\eta^2$-peroxide complex $[Fe(OEP)O_2]^-$ (**1**), which may or may not have a second axial ligand.

Table I

| complex | | | α band | 6th ligand |
|---|---|---|---|---|
| Fe(OEP)CO | 409 | 526 | 556 | pyr |
| $Fe(OEP)CCl_2$ | 408 | 531 | 560 | pyr |
| Fe(OEP)CS | 408 | 529 | 560 | pyr |
| $Fe(OEP)O_2$ | 410 | 530 | 563 | $CH_3CN$ |
| $Fe(OEP)(CO)_2$ | 409 | 536 | 566 | CO |
| $[Fe(OEP)O_2]^-$ | 425 | 545 | 572 | $Me_2SO$[a] |
| $[Fe(OEP)O_2]^-$ | 423 | 543 | 570 | $CH_3CN$[a] |

[a] The complex was prepared in the pure solvent.[15] Values in nm.

Thus three separate but isoelectronic reactions generate the same species: However, the fourth isoelectronic reaction, namely,




**1**

that of the ferric porphyrin with peroxide dianion was unsuccessful in our hands. In $Me_2SO$,. which we and others[16] find to be the best solvent for stabilizing **1**, sodium peroxide being a strong base generates $Me_2SO^-$, and upon addition in excess gives $Fe^{II}OEP$ and $Fe^{I}OEP$.

Of interest, and in contrast to the ESR data which suggest a high-spin ferric state, the optical spectrum could be rationalized in terms of a six-coordinate ferrous complex containing a very strong $\pi$-acceptor $\eta^2$-dioxygen ligand. In fact the $\eta^2$-dioxygen moiety appears to be the strongest $\pi$ acceptor so far observed. In an expression of the bathochromic sereis,[17] $CO < CCl_2 < CS < O_2 < 2CO$, the ability of an axial ligand to accept heme electron density causes a bathochromic shift of the $\alpha$ band; with $[Fe(OEP)O_2]^-$ this is red shifted 7 nm compared to $Fe(OEP)O_2$ (see Table I) indicating a high electron density on the ligand.

Although the overall negative charge will cause some red shift, it is apparent that the already weak O–O bond will be additionally weakened by the back bonding from iron to the antibonding orbitals of the dioxygen moeity; in addition, the presence of a negatively charged thiolate in the enzyme can be expected to accentuate this.[18] Assuming that the complex described here represents the electronic configuration immediately after the second enzymic reduction of oxy-P450, the facile cleavage of the O–O bond could be envisaged in two ways.

Heterolytic cleavage assisted by protonation or acylation[19] could give a ferric oxene, isoelectronic with an oxoiron(V) complex (Fe=O). The elegant work of Groves[20] on mimicking P-450-type hydroxylations by using ferric porphyrins and iodosylbenzene and the more recent characterization of an oxoporphinatochromium(V) complex[21] suggest that an oxoiron(V) intermediate may be involved in P-450 itself. Homolytic cleavage of the O–O bond could also account for hydrocarbon hydroxylations via radical intermediates, and indeed metabolism of the pesticide dieldrin by liver microsomes can be interpreted in this fashion.[22] In addition, the formation of a discrete intermediate organic substrate is strongly suggested from the studies on the hydroxylation of tetradeuterionorbornadiene.[23]

(11) Borg, D. C.; Fajer, J.; Felton, R. H.; Dolphin, D. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, 813–820.

(12) This voltage was used in order that sufficient current flowed for a rapid reduction. In this solvent system the other possible couples are −1.1 V for Fe(II) ⇄ Fe(I) and ~−1.0 V for the reduction of dioxygen. With $P\text{-}450_{cam}$ the couple for $Fe^{II}O_2 \rightleftarrows [Fe^{II}O_2]^-$ is −0.21 V which compares favorably with the −0.24 V we observe for our model system. All potentials are quoted vs. the Ag/AgCl electrode.

(13) Reduction of $[Fe^{III}(OEP)Cl]$ at −1.1 V in $CH_3CN/THF$ at room temperature generates $Fe^{I}(OEP)$ (**2**). When oxygen was admitted at room temperature formation of only the $\mu$-oxo dimer was observed. When, however, **2** was oxygenated at −50 °C, $[Fe(OEP)O_2]^-$ (**1**) was formed. These observations are similar to those of Reed et al.[14] on the corresponding tetraphenylporphyrin system in THF at −70 °C.

(14) Reed, C. A.; Mashiko, T.; Scheidt, W. R.; Haller, R. 1st International Symposium on $O_2$ Activation and Selective Oxidations Catalyzed by Transition Metals, Bendor, France, 1979; Poster Abstract.

(15) DMSO (5 mL), distilled from BaO, was deoxygenated under vacuum and dibenzo-18-crown-6 (100 mg) was added under argon. Finely divided $KO_2$ was added, in a glove box, and the solution stirred under vacuum for 2 h. This solution upon addition to $Fe^{III}(OEP)Cl$, in the complete absence of oxygen, gives initially $Fe^{II}OEP$ and then $[Fe^{I}(OEP)O_2]^-$ (**1**). These reactions and our observations exactly parallel those reported by others recently.[16]

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(23) Groves, J. T.; McClusky, G. A.; White, R. E. Coon, M. J. *Biochem. Biophys. Res. Commun.* **1977**, *81*, 154–160.

We and others[24] have examined complex **1** in order to try to mimic the reactions controlled by P-450; yet, in the attempted epoxidation of styrene, complex **1** exhibited no reactivity. We suspect that the axial thiolate ligand which is absent from our model systems may be critical and oxidations may not be observed until we can incorporate into complex **1** a good $\pi$-donor electron rich ligand.

The closely related monooxygenase, heme oxidase, which participates in the conversion of heme to bile pigments has also recently been shown to be activated by the reduction of an oxygenated ferrous heme.[25] At this time the number of electrons involved is not apparent, but since the reductant is NADPH–cytochrome *c* reductase one can assume that the electrons are added one at a time. This suggests that an intermediate such as **1** may also be involved in this system.

**Acknowledgment.** This work is a contribution from the Bioinorganic Chemistry Group and was supported by grants from the N.S.E.R.C. of Canada and the United States NIH (AM 17989).

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## Biosynthesis of Streptothricin F. 1. Observing the Interaction of Primary and Secondary Metabolism with [1,2-$^{13}C_2$]Acetate

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Streptothricin F (**1**)[2,3] is representative of a ubiquitous family of broad spectrum antibiotics[4] produced by *Streptomyces* species. All have the same unusual heterocyclic moiety, streptolidine (**2**), for which King et. al.[5] proposed the biogenesis from arginine **3** shown in Scheme I.

Subsequent biosynthetic studies by two different groups[6-9] produced conflicting results and led one group to claim[7,8] that there are two fundamentally different pathways leading to **2**: one derived from arginine and the other from acetate. [U-$^{14}$C]Arginine was used in all cases, making it impossible to determine the specificity of incorporation, much less the pathway involved. In one organism sodium [1-$^{13}$C]- and [2-$^{13}$C]acetates were specifically incorporated, but the authors were unable to propose a pathway and concluded that "Streptolidine... seems to be formed by at least two different pathways in the streptothricins".[8] We now present evidence that there is only one pathway to **2**, with **3** the direct primary precursor.

Scheme I. Biogenesis of the Streptolidine Portion of Streptothricin F from Arginine

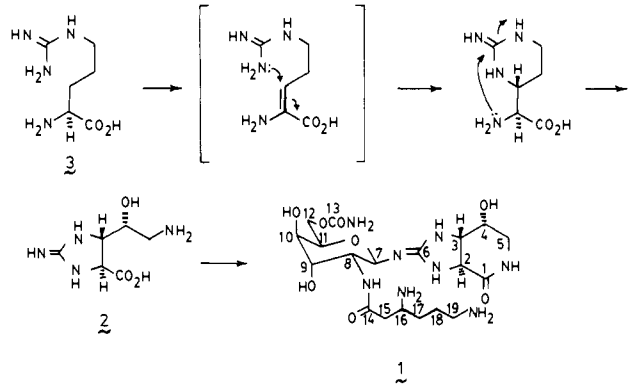

Table I. $^{13}$C NMR of Streptothricin F·3HCl[a]

| assignment | chemical shift, $\delta$[b] | $J_{CC}$, Hz | assignment | chemical shift, $\delta$[b] | $J_{CC}$, Hz |
|---|---|---|---|---|---|
| 14 | 169.7 s[c] | 48.1 | 4 | 58.5 d | 37.0 |
| 1 | 167.7 s | 55.5 | 12 | 57.9 t | |
| 6, 13 | 160.4 s | | 2 | 52.1 d | 55.5 |
| | 155.4 s | | 5 | 46.8 t | 37.0 |
| 7 | 76.5 d | | 8 | 46.6 d | |
| 9, 10, 11 | 71.1 d | | 16 | 45.9 d | 37.0 |
| | 67.6 d | | 19 | 36.6 t | |
| | 64.1 d | | 15 | 34.2 t | 48.1 |
| 3 | 58.9 d | | 17 | 26.9 t | 37.0, 35.1 |
| | | | 18 | 20.5 t | 35.1 |

[a] Bruker WH-270, 67.88 MHz; spectral width 15 000 Hz; 30° pulse angle; 0.54-s aquisition time; 66 500 transients; 48 mg in 0.5-mL $D_2O$ containing 2% pyridine. The observed signal enhancements were calculated by comparing the normalized area of each set of signals with that of the natural abundance antibiotic. [b] Middle pyridine signal = 135.5 ppm. [c] Multiplicity in gated decoupled spectrum: s = singlet, d = doublet, t = triplet.

In their [$^{13}$C]acetate feedings the Japanese group fed half the labeled material at the beginning of the fermentation and the second half at the onset of antibiotic production. Acetate, as its coenzyme A thioester, is the most central of all primary metabolites and the feeding protocol used was likely to label a large array of intermediates, interconnected by primary metabolic grids, before streptothricin biosynthesis began. These specific labelings unrelated to secondary metabolism could obscure the branch point from primary metabolism into the streptothricin pathway.

Using *Streptomyces* L-1689-23 and the same feeding protocol as the previous workers,[8] we have obtained specific incorporations of sodium [1,2-$^{13}C_2$]acetate into the streptolidine and $\beta$-lysine portions of **1**. Sodium [1,2-$^{13}C_2$]acetate (281 mg, 3.35 mmol, 90 atom %/$^{13}$C), sodium acetate trihydrate (456 mg, 3.35 mmol), and sodium [2-$^{14}$C]acetate (112 $\mu$Ci) were dissolved in 20 mL of water. To each of 4 250-mL fermentations,[10] 2.5 mL of this solution was added aseptically immediately after innoculation with a seed culture.[11] The fermentations were then shaken on a New Brunswick Rotary Shaker at 200 rpm and 29 °C. At the onset of streptothricin F production, 12 h later, the second half of the acetate mixture was divided aseptically among the four flasks, and the fermentations were continued for an additional 36 h.

Workup of the fermentations[12] eventually afforded, after re-

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(10) The medium for production fermentations consisted of corn starch, 30%; molasses, 2%; soy flour, 1.5%; and calcium carbonate, 1%. In 1-L Erlenmeyer flasks, 250-mL fermentations were run.
(11) The medium for the seed culture consisted of beef extract, 0.3%; yeast extract, 0.5%; tryptone, 0.5%; dextrose, 0.1%; and corn starch, 2.4%. In baffled 250-mL Erlenmeyer flasks, 50-mL fermentations were run.

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