chromatography (alumina, $CHCl_3$) gave semicorrin **38** as a clear colorless oil: ir ($CHCl_3$) 3300, 1730, 1650, 1590, 1520 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.02 (s, 3 H), 1.09 (s, 3 H), 1.15 (s, 3 H), 1.29 (s, 3 H), 1.37 (s, 3 H), 1.59–2.78 (m, 5 H), 2.50 (s, 2 H), 3.64 (s, 3 H), 4.88 (s, 1 H), 5.11 (b, 2 H); mass spectrum ($-H_2O$) calcd for 318.1942, found 318.1930.

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Studies on the Synthesis of Vitamin B_{12}. 2. Synthesis of the "Southern" Half

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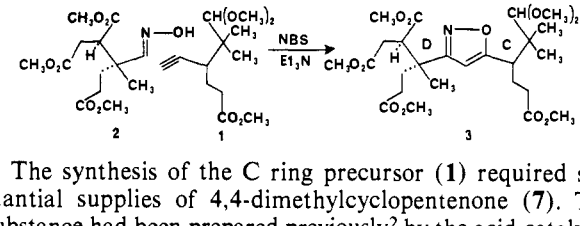
Abstract: The synthesis of racemic precursors of the C and D rings (**1** and **2**) of cobyric acid is presented together with their combination into a latent form (**3**) of the "southern" half of the vitamin.

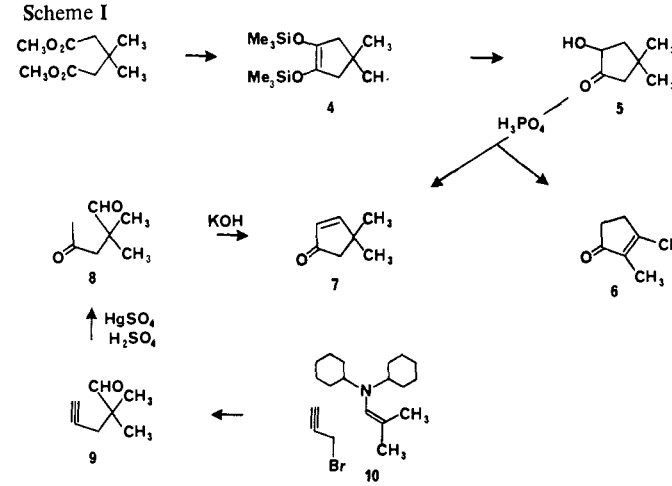
In the preceding paper[1] the close structural and stereochemical features of the "counterclockwise" precursors to the A, B, and C rings of cobyric acid were noted, and a general method of approach for their synthesis was presented together with some informative model studies. In this paper we describe the synthesis of the *racemic* forms of the C and "anomolous" D rings (**1** and **2**) and their combination into a latent form (**3**) of the "southern" half of the vitamin.



2 1 3

The synthesis of the C ring precursor (**1**) required substantial supplies of 4,4-dimethylcyclopentenone (**7**). This substance had been prepared previously[2] by the acid-catalyzed dehydration of acyloin **5** which, in turn, can be obtained directly from 3,3-dimethylglutarate or, alternatively, through the intermediacy of bis(trimethylsilyloxy)cyclopentene **4**.[3] Unfortunately, as shown more recently[4] and confirmed by others,[5] the dehydration of **5** to **7** is accompanied by substantial amounts of the rearranged cyclopentenone **6** which prompted us to investigate an alternate method (see Scheme I). Alkylation of the *N,N*-dicyclohexylenamine (**10**) of isobutyraldehyde with propargyl bromide gave aldehyde **9** as described previously.[6] The usual mercuric ion catalyzed hydration of the terminal acetylene provided ketoaldehyde **8** which suffered

Scheme I

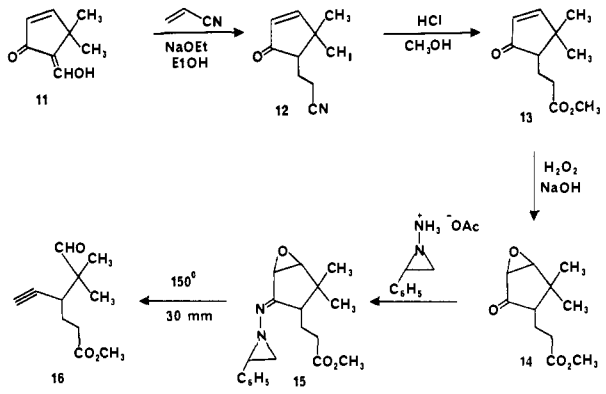


intramolecular aldol condensation when exposed to base. The overall yield for this sequence was reproducibly in the 50–60% range and allowed us to build up a substantial supply of pure enone **7**.

Attempts to alkylate **7** directly with methyl acrylate or acrylonitrile were uniformly unsuccessful. However, treatment of **7** with ethyl formate and base provided the activated α-formyl derivative **11** in high yield. Although this substance could be alkylated with methyl acrylate to provide **13** directly, it proved to be more expeditious to alkylate first with acrylo-

nitrile and to hydrolyze the resultant nitrile (**12**) with methanolic HCl (see Scheme II). Epoxidation of enone **13** with al-

Scheme II

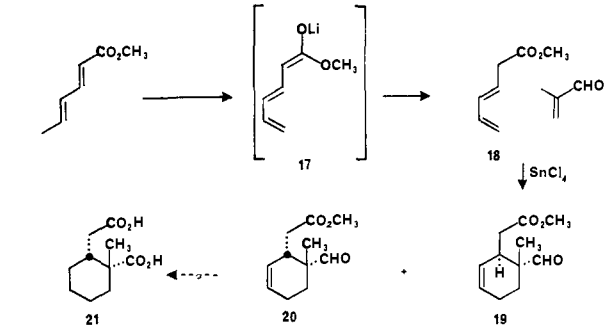


kaline hydrogen peroxide afforded **14** as a mixture of diastereomers from which hydrazone **15** was readily prepared according to the Eschenmoser procedure[7] and pyrolyzed at 150 °C to provide acetylenic aldehyde **16** in an overall yield of about 50% from enone **13** with no effort extended to maximize the conditions. Finally, protection of the aldehyde as its dimethyl acetal afforded the racemic precursor (**1**) for ring C.

The "anomolous" nature of the ring D precursor (**2**) in the "counterclockwise" approach has already been noted,[1] and necessitated an entirely different method of approach. A key step (**18** to **20**) in the elaboration of this substance takes advantage of the well-known endo stereoselectivity of the venerable Diels–Alder reaction to introduce the two chiral centers present in this intermediate in the correct *relative* sense. It should be noted that in the actual synthesis of the vitamin it will be essential for these centers to also be correct in the stereochemically *absolute* sense in order to avoid complex mixtures of diastereomers. Although there are a number of likely candidates for effecting a resolution in the sequence which follows, this was deemed unnecessary for purposes of the present investigation.

The requisite diene (**18**) was generated in high yield by quenching kinetically with acetic acid the enolate (**17**) derived from methyl sorbate (see Scheme III). The uncatalyzed cy-

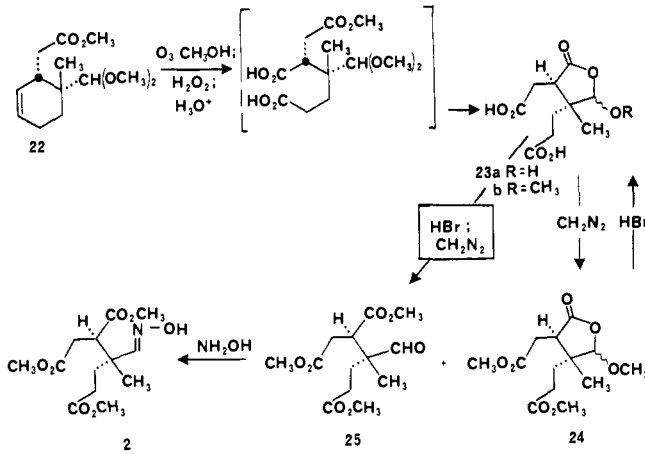
Scheme III



cloaddition of diene **18** and 2-methyl acrolein provided adducts **20** and **19** in a ratio of 83:17, respectively. The endo stereoselectivity could be increased to 91:9 by employing stannic chloride as catalyst[8] at 0–5 °C. The two isomers could be separated on a preparative scale by employing low-pressure (50 psi) liquid chromatography on silica, and the structure and stereochemistry of the major isomer (**20**) confirmed unambiguously by an unexceptional degradation[9] to the known[10] diacid **21.**

The conversion of **20** to the desired aldoxime **2** required protection of the aldehyde as its dimethyl acetal[11] (**22**). Oxidative fission of the olefin was achieved by treating **22** with ozone in methanol followed by alkaline hydrogen peroxide (see Scheme IV). After destruction of the excess peroxide, the

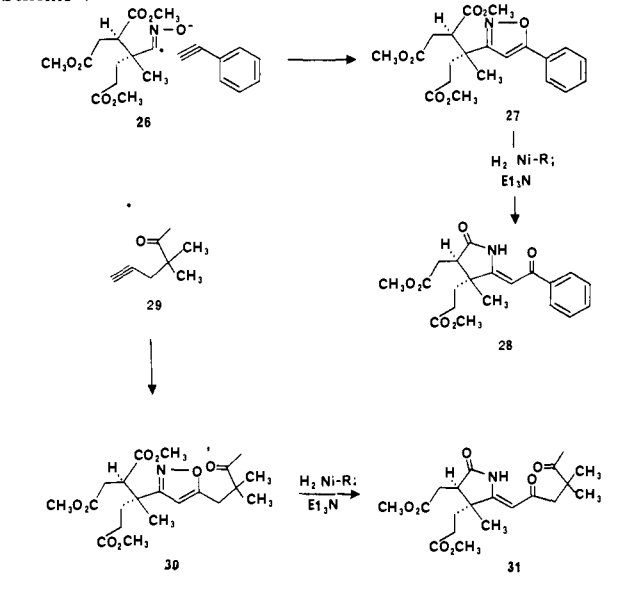
Scheme IV



methanolic solution was acidified with aqueous sulfuric acid. This sequence of reactions afforded a mixture of lactol diacid **23a** and the corresponding ether **23b.** This mixture was treated directly with diazomethane to provide diester **24** (as a mixture of two epimers) and aldehyde **25** which could be separated by low-pressure chromatography on silica. The major epimer of **24** (configuration unknown) crystallized upon standing and was fully characterized. It could be converted back to **23a** with hot hydrobromic acid. However, for preparative purposes, it was found more convenient to simply treat the crude mixture **23a** and **23b** with hot hydrobromic acid followed immediately with diazomethane. By this latter method the overall yield from **22** to **25** was a more than adequate 64%.

Since aldoxime **2** contains nearly all of the essential features found in ring D of cobyric acid, two model studies were undertaken to secure vital spectral data in anticipation of its subsequent deployment. By utilizing the NBS/Et_3N/DMF procedure aldoxime **2** was converted in situ into the corresponding nitrile oxide (**26**) and, in separate experiments, allowed to react with phenyl acetylene and ynone **29** to afford isoxazoles **27** and **30** (see Scheme V). Reduction of each of

Scheme V



these substances over a Raney nickel catalyst and subsequent treatment of the crude reaction mixtures with Et_3N to induce lactam formation gave vinylogous imides **28** and **31.** The overall yield of the **26–28** transformation was a very gratifying 77% and that for the corresponding **26–31** sequence, 79%.

Fortified with these results we turned our attention to the ultimate goal of the present investigation—isoxazole **3.** Once again, utilizing the NBS/Et_3N/DMF procedure we were

pleased to observe that **1** and **2** can be combined in the manner anticipated to provide **3**. Based on these results and those cited in the previous paper, we conclude that incorporation of the requisite side chains on the nitrile oxide and acetylenic partners has little or no effect on the formation of the required isoxazoles or on their hydrogenolysis to vinylogous amides—a problem of major concern when these studies were initiated. As gratifying and illuminating as these results have been, the transformation of **1** and **2** into **3** brings sharply into focus the third fundamental problem anticipated in the previous paper—stereochemistry. It will be noted that compound **3** contains three chiral centers or a total of eight possible stereoisomers. The endo stereoselectivity in the **18** to **20** transformation fixes the two chiral centers in oxime **2** in the correct *relative* sense reducing the number of stereoisomers in compound **3** to four. Of course, in the actual synthesis of cobyric acid, this is still an entirely unsatisfactory state of affairs. In order to achieve this objective, it is essential that each of these centers be incorporated in the correct *absolute* sense. Although many problems undoubtedly remain to be solved before we are able to reach this goal, initial experiments in our laboratory have uncovered an apparently viable solution to this remaining aspect of the problem—but, that will have to be the subject of future reports.

Experimental Section[12]

4-Oxo-2,2-dimethyl-1-pentanal (8). A magnetically stirred solution of $HgSO_4$ (1.86 g, 6.28×10^{-3} mol), concentrated H_2SO_4 (4.9 ml, 8.85×10^{-3} mol), and distilled H_2O (123 ml, 6.83 mol) was prepared under N_2. 2,2-Dimethyl-4-pentynal (**9**)[6] (34.1 g, 0.31 mol) was added dropwise to maintain the temperature below 45 °C. The mixture was allowed to stir at room temperature for 3 h and then filtered through Celite. The filtrate was extracted with 100 ml of ether. The aqueous layer was then saturated with NaCl and extracted with ether (3 × 75 mol). The combined organic layers were washed with saturated NaCl (2 × 100 ml) and dried over Na_2SO_4. Removal of the ether under reduced pressure provided 39.9 g (100% of essentially pure ketoaldehyde **8:** bp 98–99 °C (38 mm); ir (film) 1730, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.47 (s, 1 H), 2.65 (s, 2 H), 2.06 (s, 3 H), 1.10 (s, 6 H); mass spectrum *m/e* 128.

4,4-Dimethylcyclopentenone (7). A magnetically stirred solution of KOH (25 g, 0.447 mol) in H_2O (1000 ml) was degassed with N_2 at 70 °C for 4 h. Ketoaldehyde **8** was added dropwise to the hot solution and allowed to stir for 9 h at 70 °C. The mixture was cooled and extracted with ether. The aqueous layer was saturated with NaCl and extracted again with ether (3 × 150 ml). The combined ether extracts were washed with brine and dried over $MgSO_4$. Removal of the ether and distillation of the residue provided pure **7** (28.9 g, 83%), identical with a sample prepared by the known sequence **5** → **7.**

α-Formylketone 11. A mechanically stirred solution of $NaOCH_3$ (24.3 g, 0.45 mol) in ether (200 ml) was cooled to 0 °C under N_2. Ethyl formate (620 ml, 7.5 mol) was added all at once and the mixture cooled back to 0 °C. 4,4-Dimethylcyclopentenone (33 g, 0.3 mol) in ether (50 ml) was added dropwise and the mixture stirred for 13 h allowing the solution to warm to 10 °C. The cold solution was then poured into 300 ml of H_2O and extracted with ether (4 × 100 ml) to remove a small amount of unreacted ketone. The basic aqueous layer was carefully acidified at 0 °C with cold concentrated HCl to a pH of ca. 2 and extracted with CH_2Cl_2 (6 × 150 ml). The combined CH_2Cl_2 extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent left a light-yellow solid which was purified by distillation [bp 92–94 °C (3 mm)] to provide 34.8 g (84%) of pure **11:** mp 76–77 °C; ir ($CHCl_3$) 1600, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.08 (s, 1 H), 7.13 (s, 1 H), 7.15, 6.18 (AB q, 2 H), 1.29 (s, 6 H); mass spectrum *m/e* 138.

Nitrile 12 and Ester 13. To a magnetically stirred solution of NaOEt in EtOH (2.79 g of Na, 12.1 mmol, 450 ml) under N_2 was added 59.8 g (0.404 mol) of formyl ketone **11** followed by dropwise addition of acrylonitrile (107 ml, 1.62 mol). The mixture was maintained at room temperature for 1.5 h and then refluxed for 20.5 h. After cooling the solvent and excess acrylonitrile was removed, and the residue was diluted with ether (400 ml) and extracted with 4% NaOH (4 × 200 ml) to remove starting material. The ether layer was washed with brine and dried over $MgSO_4$. Removal of the ether and distillation provided pure **12** (30.8 g, 48%): bp 100–102 °C (1.5 mm); ir (film) 2240, 1680, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35, 5.91 (AB q, 2 H), 2.84–1.69 (m, 5 H), 1.26 (s, 3 H), 1.02 (s, 3 H). This material was used directly in the next step.

Nitrile **12** (14.6 g, 9.2 mmol) and H_2O (1.99 ml, 11 mmol) were dissolved in 500 ml of CH_3OH, and the solution was saturated with dry HCl. The bright red solution was refluxed for 6.5 h. The methanol was removed and 250 ml of H_2O added to the residue. The mixture was extracted with ether (5 × 100 ml), and the organic layers were washed with saturated $NaHCO_3$ (2 × 300 ml), followed by brine and dried over $MgSO_4$. Removal of the solvent and distillation gave pure ester **13** (12.8 g, 63%): bp 89–91 °C (3 mm); ir (film) 1617, 1710, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43, 6.03 (AB q, 2 H), 3.68 (s, 3 H), 2.90–1.60 (m, 5 H), 1.25 (s, 3 H), 1.08 (s, 3 H); mass spectrum *m/e* 196.

Epoxide 14. Enone **13** (2.1 g, 11 mmol) was dissolved in 8 ml of methanol and 3.2 ml of 30% H_2O_2 added. The solution was cooled in an ice bath and 1 ml of 6 N NaOH added dropwise. The mixture was then stirred at room temperature for 7 h, then poured into 150 ml of H_2O and extracted with ether. The organic phase was washed with brine and dried over $MgSO_4$. Removal of the solvent and distillation provided 1.8 g (82%) of pure epoxide: bp 115 °C (3 mm); ir ($CHCl_3$) 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.67 (s, 3 H), 3.43 (AB q, 2 H), 1.30 (s, 3 H), 0.90 (s, 3 H); mass spectrum *m/e* 212.

Hydrazone 15. Epoxide **14** (1.06 g, 0.5 mmol) was dissolved in 25 ml of CH_2Cl_2 and the solution cooled to 0 °C under N_2. 2-Phenyl-1-aminoaziridinium acetate (1.07 g, 0.55 mmol) was added all at once and the solution stirred for 3.5 h at 0 °C. The mixture was then washed with saturated $NaHCO_3$ (2 × 15 ml) and dried ($MgSO_4$). Removal of the CH_2Cl_2 in vacuo left 1.56 g (95%) of essentially pure **15,** which was used directly in the next step: ir (film) 1600, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.32 (s, 5 H), 6.25, 6.38 (dd, 1 H), 6.68 (s, 3 H), 6.30 (d, 1 H); mass spectrum *m/e* 328.

Acetylenic Aldehyde 16. Hydrazone **15** (1.0 g, 0.258 mmol) was placed in the last bulb of a four-bulb Kugelrohr set-up with an equal volume of glass beads. The last two bulbs were then placed in the oven in vacuo (30 mm) at 150 °C. Pure ynone **16** (318 mg, 63%) collected in the first bulb out of the oven: ir (film) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.56 (s, 1 H), 3.61 (s, 3 H), 2.78–1.67 (m, 6 H), 1.08 (s, 3 H), 1.03 (s, 1 H); mass spectrum calcd for 196.1099, found 196.1096.

Acetylenic Acetal 1. A mixture of trimethyl orthoformate (2.88 ml, 2.63 mmol), anhydrous methanol (0.75 ml, 1.84 mmol), $TsOH \cdot H_2O$ (2.52 mg), and aldehyde **16** was refluxed for 4 h under N_2. After cooling, the mixture was diluted with ether (10 ml) and washed with saturated $NaHCO_3$ (2 × 10 ml), followed by brine (10 ml) and dried over Na_2SO_4. Removal of the ether and distillation in the Kugelrohr apparatus yielded 591 mg (93%) of pure acetal **1:** bp 85–88 °C (1.0 mm); ir (film) 3270, 2840, 2815, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.14 (s, 1 H), 3.63 (s, 3 H), 3.48 (s, 6 H), 2.81–2.29 and 1.92–1.47 (m, 5 H), 2.08 (d, 1 H, J = 2 Hz), 0.96 (s, 3 H), 0.92 (s, 3 H); mass spectrum *m/e* 242.

Methyl 3,5-Hexadienoate 18. A solution of lithium diisopropylamide (LDA) in THF was prepared by the slow addition of *n*-BuLi (0.55 mol, 2.2 M in hexane) in 250 ml of hexane to a solution of diisopropyl amine (55.6 g, 0.55 mol) in 1000 ml of dry THF under N_2. The solution was cooled to −78 °C, and dry hexamethyl phosphoramide (117.4 g, 0.65 mol) was added dropwise and the mixture allowed to stir 0.5 h. Methyl sorbate (63 g, 0.5 mol) was added to the cold solution over 2 h. Stirring was continued an additional hour at −78 °C, then the dark-red mixture was siphoned into a rapidly stirred solution of acetic acid (90 g, 1.5 mol) in 1.8 l. of H_2O. The resultant solution was extracted with pentane (5 × 200 ml), and the combined extracts were washed with water, 1 N $NaHCO_3$, dried (Na_2SO_4), and concentrated by distillation through a 10-in. column packed with helices. The residue was distilled to give pure diene **18** (50.8 g, 82%): bp 73–75 °C (20 mm); ir (film) 1740, 1250 cm^{-1}; ^1H NMR (CCl_4) δ 3.04 (d, J = 6 Hz, 2 H), 3.60 (s, 3 H), 5.8–6.5 (m, 5 H); mass spectrum *m/e* 126.

Diels–Alder Adducts 19 and 20. Stannic chloride pentahydrate (32.2 g, 0.092 mol) was dissolved in diene **18** (57.8 g, 0.46 mol) at room temperature and then cooled to 0 °C under N_2. Freshly distilled methacrolein (161 g, 2.3 mol) cooled to −20 °C was added to the solution and the mixture kept at 0–5 °C for 2 days. At the end of this time the mixture was neutralized with 2 N Na_2CO_3 and extracted with

ether. The extracts were washed with H_2O, brine, dried ($MgSO_4$), and concentrated under reduced pressure. Distillation afforded 54.7 g (61%) of adducts **19** and **20**, bp 78–81 °C (0.2 mm), in a ratio of 1:10, respectively. The ratio was determined by integration of the signals at δ 10.0 (major) and 9.8 (minor). The mixture could be separated by preparative GLC on 8 ft × ¼ in. 15% FFAP on 60–80 Chromosorb P at 200 °C or more conveniently for preparative purposes by medium pressure (50 psi) liquid chromatography on silica ($CHCl_3$:ethyl acetate 85:15). Ir (film) 2860, 2740, 1740, 1170 cm^{-1}; ^1H NMR (CCl_4) δ 1.13 (s, 3 H), 1.3–2.8 (br m, 7 H), 3.65 (s, 3 H), 5.5–5.8 (m, 2 H) and 10.0 (s, 1 H); mass spectrum calcd for 196.1099, found 196.1100. An oxime derivative was prepared, mp 85–87 °C.

Ester Acetal 22. Aldehyde **20** (33.1 g, 0.17 mol), trimethyl orthoformate (180 g, 1.7 mol), and dry methanol (16.3 g, 0.51 mol) were combined and stirred under N_2. This mixture was treated with TsOH (1.6 g, 8.5 mmol) and allowed to stir for 18 h. At the end of this time the volatile components were removed under reduced pressure, and the residue was poured into 1 N $NaHCO_3$ and extracted with ether (3 × 100 ml). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was distilled through a 4-in. column packed with helices initially at 1–5 mm to remove traces of trimethyl orthoformate and then at 0.3 mm to provide 41.0 g (100% of pure acetal (bp 92–94 °C): ir (film) 1740, 1160, 1110, 1070 cm^{-1}; ^1H NMR (CCl_4) δ 0.94 (s, 3 H), 1.2–1.6 (m, 2 H), 1.7–2.6 (m, 5 H), 3.44 (s, 3 H), 3.48 (s, 3 H), 3.64 (s, 3 H), 3.94 (s, 1 H), 5.60 (m, 2 H); mass spectrum ($-CH_3OH$) calcd for 210.1256, found 210.1246.

Aldehyde 25. Acetal **22** (1.21 g, 5 mmol) was dissolved in 50 ml of CH_3OH in a gas washing bottle and cooled to −78 °C. Ozone was passed through the solution (1.4 meqiv/min) until a 50% excess had been used and the solution was then purged with N_2 for 30 min. The cold solution was added to a mixture of NaOH (1 g, 24 mmol) and 30% H_2O_2 (1.7 g, 14.5 mmol) in 20 ml of H_2O. After stirring at room temperature for 24 h, a small amount of Pd/C was added to destroy excess peroxide. The catalyst was removed by filtering through Celite. Water (20 ml) was added and the solution adjusted to pH 1 with concentrated H_2SO_4. The solution was refluxed for 30 h and then the CH_3OH removed under reduced pressure. The residue was then extracted with ether and the organic layer dried over $MgSO_4$ and then concentrated to afford 1.15 g of lactols **23a** and **23b**. The crude mixture was treated with 48% HBr (30 ml) at 80 °C for 24 h to convert **23b** into **23a**. After cooling, 30 ml of ice water was added and the solution extracted with 75 ml of ether to remove impurities. Continuous extraction of the acidic solution with ether for 2 days afforded a yellow gummy solid (**23a**) which was treated with excess diazomethane to provide 924 mg (64%) of pure aldehyde **25**: bp 163–165 °C (0.25 mm); ir (film) 2860, 2740, 1735 cm^{-1}; ^1H NMR (CCl_4) δ 1.0 (s, 3 H), 1.6–3.25 (complex m, 7 H), 3.56 (s, 3 H), 3.60 (s, 6 H), 9.7 (s, 1 H); mass spectrum *m/e* 288 (M − 1 287 larger).

Lactol Ether 24. The crude mixture of **23a** and **23b** described above was treated with excess diazomethane in the usual way. Chromatography on alumina (activity III) with $CHCl_3$:hexane (85:15) provided lactol ether **24** as a mixture of epimers in an approximately 2:1 ratio. Upon standing the major isomer crystallized. It was recrystallized from EtOH–H_2O: mp 69–71 °C; ir (KBr) 1780, 1740, 1200, 1160, 1120, 930 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (s, 3 H), 1.6–2.6 (br m, 6 H), 3.10 (d, J = 8 Hz, 1 H), 3.46 (s, 3 H), 3.64 (s, 3 H), 3.68 (s, 3 H), 4.79 (s, 1 H); mass spectrum *m/e* ($-OCH_3$) 257; parent 288 (very small). Treatment of the epimeric mixture with HBr followed by diazomethane as described above gave pure aldehyde **25**.

Aldoxime 2. A solution of aldehyde **25** (1.70 g, 5.9 mmol) in 12 ml of dry pyridine was treated with NH_2OH·HCl (0.44 g, 7.1 mmol) and stirred at room temperature for 18 h. The mixture was then poured into 50 ml of ice water, brought to pH 1 with concentrated HCl, and extracted with ether (4 × 50 ml). The ether extracts were washed with brine and dried ($MgSO_4$). Removal of the solvent under vacuum left 1.62 g (90%) of oxime which was pure by TLC: ir (film) 3450 (broad), 1730 cm^{-1}; ^1H NMR (CCl_4) δ 1.1 (s, 3 H), 1.5–1.9 (br m, 2 H), 2.1–2.9 (br m, 5 H), 3.6 (s, 6 H), 3.62 (s, 3 H), 7.15 (s, 1 H), 8.2 (br s, 1 H); mass spectrum calcd for 303.1318, found 303.1323.

Isoxazole 27. Aldoxime **2** (1.09 g, 3.6 mmol) was dissolved in 15 ml of dry DMF and cooled to 4–5 °C. A solution of recrystallized NBS (0.96 g, 5.4 mmol) in 15 ml of dry DMF was added dropwise producing a golden-yellow solution. After addition was complete, the mixture was stirred in the cold for 1 h, and then a mixture of phenyl acetylene (1.84 g, 18 mmol) and dry Et_3N (0.55 g, 5.4 mmol) was added dropwise over 30 min. The golden color faded with the addition, and the mixture was stirred at room temperature for 24 h, then poured in 60 ml of water and extracted with $CHCl_3$. The extracts were washed with H_2O, brine, and dried ($MgSO_4$). Removal of the solvent and column chromatography on silica (70 g) eluting with $CHCl_3$ removed phenyl acetylene and starting aldoxime **2** (66 mg). Further elution with $CHCl_3$:CH_3OH (95:5) afforded pure isoxazole **27** (1.12 g, 90%): ir (CCl_4) 1740, 3480, 1610, 1590, 1570, 1165 cm^{-1}; ^1H NMR (CCl_4) δ 1.40 (br s, 3 H), 1.9–2.3 (m, 4 H), 2.4–3.3 (m, 3 H), 3.60 (s, 9 H), 6.45 (s, 1 H), 7.3–7.5 (m, 3 H), 7.6–7.8 (m, 2 H); mass spectrum calcd for 403.1631, found 403.1627.

Vinylogous Imide 28. Isoxazole **27** (0.2 g, 0.5 mmol) in methanol was reduced at atmospheric pressure over a W-2 Raney nickel catalyst which had been washed with acetic acid to a pH ca. 7. After the theoretical amount of H_2 was consumed, the catalyst was filtered off through Celite and washed with methanol. Several drops of Et_3N was added to induce cyclization and the solution stirred for 24 h. Removal of the solvent under reduced pressure and preparative layer chromatography (silica gel, 7:3 ethyl acetate:hexane) provided vinylogous imide **28** as a clear oil (160 mg, 86%): ir (CCl_4) 3280, 1740, 1650, 1585, 1565, 1255, and 1150 cm^{-1}; ^1H NMR (CCl_4) δ 1.23 (s, 3 H), 1.7–3.1 (br m, 7 H), 3.60 (s, 3 H), 3.70 (s, 3 H), 6.10 (s, 1 H), 7.3–7.5 (m, 3 H), 7.85–8.0 (m, 2 H); mass spectrum *m/e* 373.

Isoxazole 30. The procedure was the same as for isoxazole **27.** The quantities employed were oxime **2** (1.3 g, 4.3 mmol), NBS (1.16 g, 6.5 mmol), acetylenic ketone **29** (2.73 g, 22 mmol), and Et_3N (0.66 g, 6.5 mmol). The product was purified by preparative layer chromatography (silica gel, 50:50 ethyl acetate:hexane). The yield was 1.67 g (92%) of a viscous oil: ir (CCl_4) 1740, 1710, 1600, 1170 cm^{-1}; ^1H NMR (CCl_4) δ 1.18 (s, 6 H), 1.32 (s, 3 H), 1.8–2.3 (br m, 3 H), 2.10 (s, 3 H), 2.4–2.7 (m, 2 H), 2.8–3.1 (m, 4 H), 3.65 (s, 9 H), 5.90 (s, 1 H); mass spectrum calcd for 425.2049, found 425.2037.

Vinylogous Imide 31. The procedure was the same as for compound **28.** The product was purified by column chromatography on neutral alumina (activity III) eluting with $CHCl_3$ to provide vinylogous imide **31** as a viscous oil (86%): ir (CCl_4) 3300, 1740, 1710, 1665, 1590 cm^{-1}; ^1H NMR (CCl_4) δ 1.20 (s, 6 H), 1.7–2.5 (m, 5 H), 2.5 (s, 3 H), 2.55–3.05 (m, 4 H), 3.60 (s, 3 H), 3.66 (s, 3 H), 5.35 (s, 1 H); mass spectrum calcd for 395.1992, found 395.1987.

Isoxazole 3. Oxime **2** (107 mg, 3.53×10^{-4} mol) was dissolved in 5 ml of dry DMF and cooled to 5 °C under N_2. A solution of recrystallized NBS (94.4 mg, 5.3×10^{-4} mol) in 3 ml of DMF was added dropwise to the cooled solution and the mixture was stirred at 5 °C for 1 h. Then acetal **1** (171 mg, 7.06×10^{-4} mol) dissolved in 171 mg (5.3×10^{-4} mol) of Et_3N was added dropwise after which the mixture was allowed to warm to room temperature and stirred for 4 days. The mixture was then poured into 15 ml of H_2O and continuously extracted with ether for 24 h. After drying over Na_2SO_4 the ether was removed and the residue distilled in a Kugelrohr apparatus at 85–88 °C (1.0 mm) to remove excess acetal **1.** The crude product remaining behind was chromatographed on a preparative layer plate (silica) using ethyl acetate:hexane (50:50) as eluent to provide 134 mg (75%) of isoxazole **3** as a viscous oil: ir (film) 1593, 1741 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.28 (s, 1 H), 4.41 (br s, 1 H), 3.56 (s, 12 H), 3.42 (s, 6 H), 2.95–2.00 (m, 12 H), 1.36 (s, 3 H), 0.99 (s, 3 H), 0.83 (s, 3 H); mass spectrum calcd for 543.2679, found 543.2673.

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References and Notes

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Biosynthesis of Shihunine in *Dendrobium pierardii*[1]

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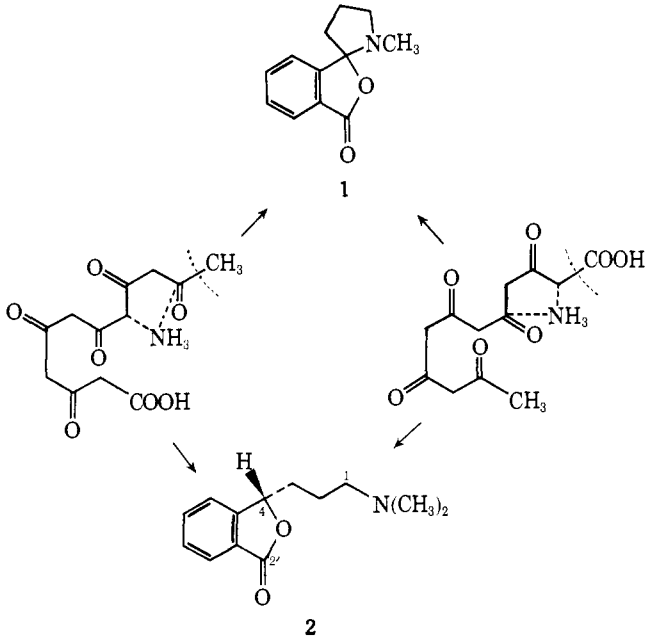
Abstract: The administration of [1-^{14}C]acetate to *Dendrobium pierardii* afforded radioactive shihunine. A systematic degradation of this alkaloid established that it was preferentially labeled at C-5, indicating that shihunine is not a polyketide. [*4,2′-carbonyl*-$^{14}C_2$]-*o*-Succinylbenzoic acid was found to be an excellent precursor of shihunine (14.4% absolute incorporation), and degradations indicated that all the activity was equally divided between C-2 and C-12. Conformation that *o*-succinylbenzoic acid is a direct precursor of shihunine was obtained by feeding this precursor labeled with both ^{13}C and ^{14}C at C-1. By the use of ^{13}C NMR it was established that the resultant shihunine was enriched only at C-5 (4.4% specific incorporation).

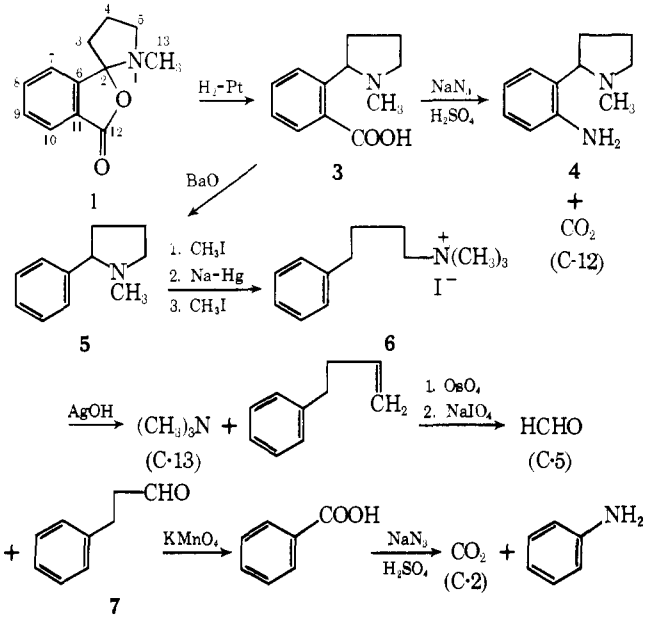
Shihunine (**1**) is a phthalidopyrrolidine, no other representatives of this class of alkaloid being known. It was first isolated from the orchid *Dendrobium lohohense*[2] by Inubushi and co-workers, who deduced its structure,[3] which has been confirmed by synthesis.[4,5] Later it was isolated from *Dendrobium pierardii*[6] which also yielded the related alkaloid pierardine (**2**).[7]

We considered that these alkaloids would have a common biosynthetic origin and initially regarded them as polyketides, even though a completely satisfying biosynthetic scheme could not be constructed (Scheme I). Nevertheless, we fed [1-^{14}C]acetate to *D. pierardii* in preliminary experiments, and labeled shihunine was isolated from the plants. A higher incorporation of activity into shihunine was obtained when the plant was fed by painting the leaves with a solution of the tracer, compared with the wick feeding method (Table I). The

Scheme I. Hypothetical Formation of Shihunine and Pierardine from a Polyketide



Scheme II. Degradation of Shihunine



shihunine was degraded as illustrated in Scheme II. Hydrogenation in the presence of Adams catalyst afforded dihydroshihunine (**3**).[3] A Schmidt reaction on this amino acid yielded 2-(2′-aminophenyl)-1-methylpyrrolidine (**4**) and carbon dioxide. On heating dihydroshihunine with barium oxide, 1-methyl-2-phenylpyrrolidine (**5**) was obtained. An Emde reduction on the methiodide of **5** afforded 1-dimethylamino-4-phenylbutane, which was converted to its methiodide **6**. A Hofmann elimination of **6** afforded trimethylamine, collected and assayed as tetramethylammonium iodide. The 4-phenyl-1-butene also obtained in this reaction was oxidized with osmium tetroxide and sodium metaperiodate yielding formaldehyde, collected as its dimedone derivative, and 3-phenylpropanal (**7**), collected as its semicarbazone. Oxidation of this semicarbazone or 1-dimethylamino-4-phenylbutane with permanganate yielded benzoic acid which was subjected to a Schmidt reaction yielding aniline (assayed as benzanilide) and carbon dioxide. The results of this degradation on the