Anodic Cyclization–Rearrangement of Methoxybibenzyls to Dihydrophenanthrones[1]

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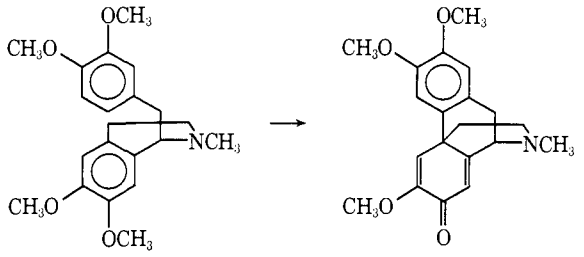
Abstract: Anodic oxidations were performed in acetonitrile at platinum using controlled potentials. 2-Methyl-3′,4,4′,5-tetramethoxybibenzyl was converted in 98% yield to 9,10-dihydro-10a-methyl-2,6,7-trimethoxy-3(10a*H*)-phenanthrone. 2-Methyl-2′,4,5′,5-tetramethoxybibenzyl produced 9,10-dihydro-10a-methyl-2,5,8-trimethoxy-3(10a*H*)-phenanthrone in 88% yield. 9,10-Dihydro-10a-methyl-2-ethoxy-6,7-dimethoxy-3(10a*H*)-phenanthrone was correspondingly formed from 2-methyl-3′,4′,5-trimethoxy-4-ethoxybibenzyl. 2-Methyl-4,5-dimethoxybibenzyl did not cyclize under these conditions. A mechanism rationalizing these products as well as cyclic voltammetric data is proposed.

The anodic coupling of dimethoxybenzene rings has recently been demonstrated to have utility and some generality. We have produced a variety of morphinandienones by this route,[2] *i.e.*

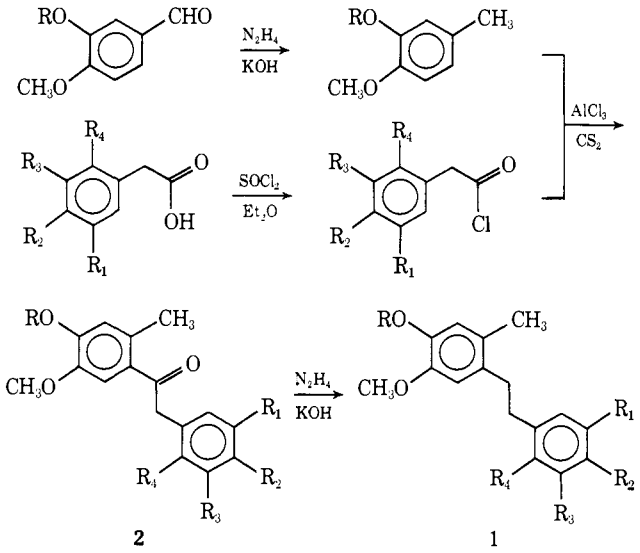


and the cyclization of an isochroman-3-one derivative has been reported.[3] In each case the cyclized product can be isolated in about 50% yield. Cyclizations to produce tetramethoxydihydrophenanthrene cation radicals have also been reported by Parker and coworkers.[4] Since all of these cyclizations proceeded without significant rearrangement to produce the phenanthrene ring system, it seemed that this approach might be applicable to the synthesis of steroids. In pursuit of this goal, we attempted to form the A, B, and C rings with the proper angular methyl group by oxidizing compounds **1**.

Results and Discussion

Synthesis. Bibenzyls **1a**, **1b**, and **1c** were prepared as shown in Scheme I. The yields were quite satisfactory. In the preparation of **1a**, for example, 3,4-dimethoxytoluene was prepared in 75% yield from commercial veratraldehyde by the method of Bruce and Sutcliffe.[5] 3,4-Dimethoxyphenylacetyl chloride was prepared from commercial 3,4-dimethoxyphenylacetic acid and added to a stirred mixture of 3,4-dimethoxytoluene and $AlCl_3$ in CS_2. After reaction, the previously unknown 4,5-dimethoxy-2-(3,4-dimethoxyphenylacetyl)toluene was isolated in 77% yield (based upon the 3,4-dimethoxyphenylacetic acid) and converted by a modified Wolff–Kishner reaction to **1a** in 82% yield. These reactions were performed on an approximately 0.1 *M* scale and no attempt was made to maximize yields.

Scheme I



a, $R_1 = R_4 = H$; $R_2 = R_3 = OCH_3$; $R = CH_3$
b, $R_1 = R_4 = OCH_3$; $R_2 = R_3 = H$; $R = CH_3$
c, $R_1 = R_4 = H$; $R_2 = R_3 = OCH_3$; $R = CH_2CH_3$

For the preparation of 2-methyl-4,5-dimethoxybibenzyl (**1d**), 4-methylveratrole was subjected to the Vilsmeier–Haack reaction to give 6-methylveratraldehyde. Addition of excess benzylmagnesium chloride gave a quantitative yield of the α-hydroxybibenzyl. An attractive and direct route to **1d** would then require reductive cleavage of the benzyl alcohol to the saturated methylene. Unfortunately, numerous attempts to perform this reaction using a variety of catalysts and conditions yielded only unchanged starting material. The alcohol, was, therefore, converted to the corresponding chloride by treatment with thionyl chloride in ether. LiAlH reduction at room temperature gave **1d** in 37% over-all yield. If the final reduction with LiAlH was performed at reflux, a white solid whose spectroscopy was consistent with a *trans*-stilbene structure was isolated in addition to **1d**. Hydrogenation over Adam's catalyst converted the white solid into **1d** in high yield.

(1) This work was supported by NIH research grant GM 19234 from the National Institute of General Medical Sciences.

(2) L. L. Miller, F. R. Stermitz, and J. R. Falck, *J. Amer. Chem. Soc.*, **93**, 5941 (1971); **95**, 2651 (1973); submitted for publication.

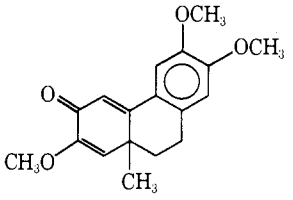
(3) M. Sainsbury and R. F. Shinazi, *J. Chem. Soc., Chem. Commun.*, 718 (1972).

(4) (a) A. Ronlan and V. D. Parker, *J. Chem. Soc., Chem. Commun.*, 1567 (1970); (b) V. D. Parker, *et al.*, Abstracts of the 166th National Meeting of the American Chemical Society, Chicago, Ill., August 1973, ORGN-83.

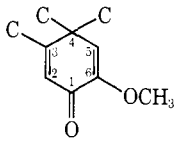
(5) J. M. Bruce and F. K. Sutcliffe, *J. Chem. Soc.*, 3824 (1956).

Preparative Oxidations. **1a** was oxidized at 0.90 V in acetonitrile at 0° with lithium perchlorate as background electrolyte in the three-compartment cell described previously.[2] The oxidation proceeded smoothly in either the presence or absence of solid sodium carbonate with no detectable change in yield. A red species could be observed streaming from the surface of the anode, eventually turning the entire solution deep red. At the end of the oxidation, the anolyte remained highly colored for days or until water was added, at which time it immediately turned pale yellow. The anolyte was then evaporated to near dryness (caution, the product mixture may contain perchloric acid), mixed with more water, and extracted repeatedly with chloroform. The chloroform extracts yielded only one product, in 98% yield, mp 205–206°. Its assignment to structure **3a** was based on its spectroscopic characteristics. The $C_{18}H_{20}O_4$ molecular formula was indicated by mass spectroscopy and verified by combustion analysis. The ir spectrum indicated the presence of a cross-conjugated α-methoxycyclohexadienone system:[6] ν_{max} (Nujol mull) 1640, 1625, and 1605 cm^{-1}. This was corroborated[6] by prominent ions at *m/e* 299 (M − 1) and 257 (M − 43) in the mass spectrum. Nuclear magnetic resonance spectroscopy (δ in $CDCl_3$) showed a tertiary methyl at 1.28 (s, 3 H); one-half of an A_2B_2 system at 1.70–2.10 (m, 2 H); the other half at 2.82–3.15 (m, 2 H); two methoxy resonances at 3.78 (s, 3 H, vinyl ether) and 3.95 (s, 6 H, aromatic methoxys); two olefinic protons at 5.82 (s, 1 H, α proton of α,β-unsaturated ketone system) and 7.13 (s, 1 H, α proton of α,β-unsaturated ketone system); and two aromatic protons at 6.63 (s, 1 H) and 6.70 (s, 1 H).

It is known[7] that signals corresponding to two protons on a substituted cyclohexadienone ring show appreciable spin–spin coupling when they are located in any of the following arrangements: (a) on adjacent carbon atoms, (b) on positions 2 and 6, (c) on positions 3 and 5 (see **4**). The absence of such coupling in the



3a



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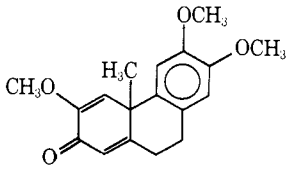

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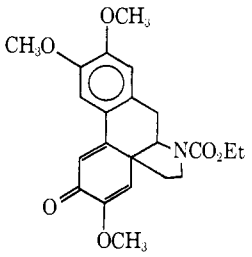
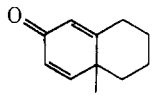


6

spectrum, assuming no skeletal rearrangement, indicated the partial structure **4**. The appearance of the aromatic protons as two singlets indicated cyclization to the other ring occurred at the least-hindered position, *para* to a methoxy group. Furthermore, the methyl group must be in the 4-position of the dienone ring since it is uncoupled and not shifted downfield as it would be if in an allylic position.

Two structures consistent with these data are **3a** and **5**. Compound **5** would be formed by simple cyclization and loss of the methoxy methyl in analogy with earlier examples.[2–4] Neither the nmr or uv–visible spectra are, however, in accord with this structure. Thus, the absorption spectrum shows several bands with a longest wavelength λ_{max} 357 nm. Methoxycyclohexadienones do not have bands beyond 300 nm.[2,7] On the other hand, β-arylcyclohexadienones should absorb at longer wavelengths. Indeed, the uv spectra of the product and model compound **6**[8] are extremely similar throughout: **3a**, λ_{max}^{EtOH} (log ϵ) 238 (4.33), 265 (4.35), 292 (4.26), 357 (4.41); **6**, λ_{max}^{EtOH} (log ϵ) 236 (4.08), 265 (4.07), 291 (3.79), 359 (3.99). The nmr spectrum of **5** should show allylic coupling between the vinyl proton at position 1 and the methylene protons at carbon 10. This coupling is seen in the model compound **7**.[9] On the other hand,



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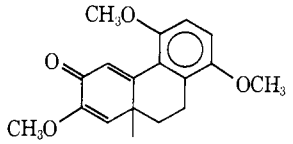
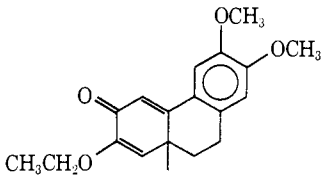


3b



3c

no allylic coupling should be seen from **3a** in agreement with the spectrum.

Electrolysis of **1b** at 0.86 V at 0° gave only one product, mp 184–185.5°, in 88% yield. As above, a persistent red species was generated which readily discharged to pale yellow when water was added. With a similar foundation as above, the product was assigned to structure **3b**. The oxidation of **1c** produced only **3c**, again in very high yield.

Thus an efficient synthesis of dihydrophenanthrones has been revealed. This reaction was originally conceived as a route to the A, B, and C rings of steroids. The unexpected coupling–rearrangement (see below) produced instead a ring system that could be used in the synthesis of the B, C, and D rings by contraction of the dienone ring. Most importantly, the requisite angular methyl is in place.

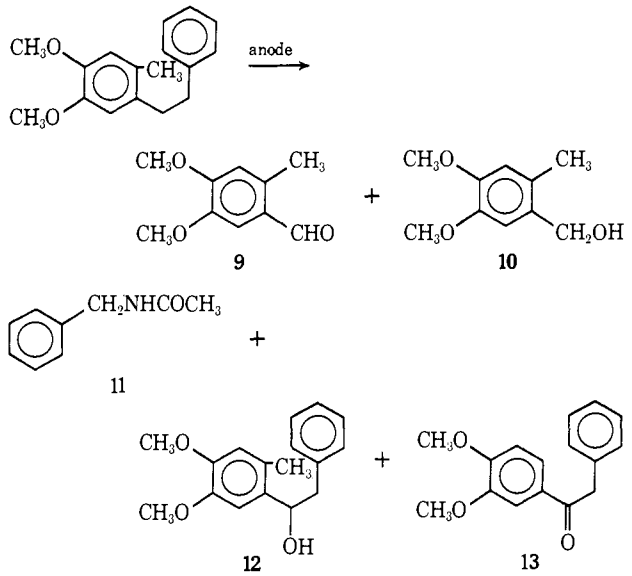
Preparative electrolysis of **1d** at 0.90 V and 0° (until coulometry indicated 2 faradays/mol of current had been passed) gave a complex product mixture. Although a red colored species could be seen streaming from the anode surface, the reaction mixture quickly turned dark brown and remained so throughout the oxidation and work-up procedure. The product mixture was chromatographed on silica gel to give unreacted starting material, 6-methylveratraldehyde (**9**),

(6) K. L. Stuart, *Chem. Rev.*, **71**, 47 (1971); T. Kametani, *et al.*, *J. Chem. Soc. C*, 624 (1970); T. Kametani, H. Sugi, S. Shibuya, and K. Fukumoto, *Tetrahedron*, **27**, 5375 (1971).

(7) A. R. Battersby, *et al.*, *J. Chem. Soc., Perkin Trans. 1*, **14**, 1736, (1972), and references cited therein.

(8) S. M. Kupchan, A. J. Liepa, V. Kameswaran, and R. F. Bryan, *J. Amer. Chem. Soc.*, **95**, 6861 (1973). We thank Professor Kupchan for communicating these results to us before publication.

(9) D. Caine, P. F. Brake, J. F. DeBardelen, and J. B. Dawson, *J. Org. Chem.*, **38**, 967 (1973).



6-methylveratryl alcohol (**10**), benzylacetamide (**11**), and 2-methyl-4,5-dimethoxy-α-hydroxybibenzyl (**12**). These products were identified by spectral comparison with authentic samples. In addition, a small amount of yellow oil was isolated and assigned structure **13**, based on its spectral characteristics. The ir spectrum indicated an aryl ketone: λ_{max} (neat) 1675 cm^{-1}. Nmr showed an aryl methyl resonance at 2.42 (s, 3 H), one methoxy resonance at 3.72 (s, 6 H), a singlet at 4.15 (2 H) in the correct region for a methylene between a phenyl and a carbonyl, and three aromatic singlets at 6.65 (1 H), 7.21 (5 H), and 7.40 (1 H). Structure **13** was confirmed by reduction of the electrolysis product by sodium borohydride in methanol to give a yellow oil whose infrared spectrum was superimposable on that of **12**.

Cyclic Voltammetry. Voltammograms were recorded using an acetonitrile–lithium perchlorate electrolyte solution. A two compartment cell was employed in which the Ag|$AgNO_3$ reference electrode was separated from the platinum anode and the cathode by a glass frit. Model compound 4,5-dimethylveratrole showed a quasireversible couple centered at 0.85 V (Figure 1). The peak separation ($E_{P_a} - E_{P_c}$) was 75 mV at a scan rate of 500 mV/sec. This demonstrates that the cation radical has a lifetime greater than 1 sec. Similar behavior was noted for **1d** where $E_{P_a} - E_{P_c}$ = 80 mV. Compounds **1a** and **1b** in contrast did not show any cathodic peak on the reverse sweep (see Figure 1) demonstrating that these cation radicals are unstable, on this time scale. E_{P_a} values are reported in Table I. The similarity of the first peak potentials for **1a**, **1b**, **1d** and 4,5-dimethylveratrole suggests that initial transfer involves the tetrasubstituted ring. The E_{P_a} of 4-methylveratrole (**2**) is, however, close enough so that this is not a firm conclusion. Indeed, one-electron oxidation of both rings may precede coupling.[4b] Comparison of the E_{P_a} for the anodic product **3a** and **1a** demonstrates that the dienone oxidizes somewhat more difficultly than the bibenzyl and hence overoxidation can be avoided with controlled potentials.

Mechanism. The coupling reaction is very clean but unexpectedly leads to rearranged products. The data available are limited but do allow a mechanistic hypothesis to be formulated as indicated in Scheme II

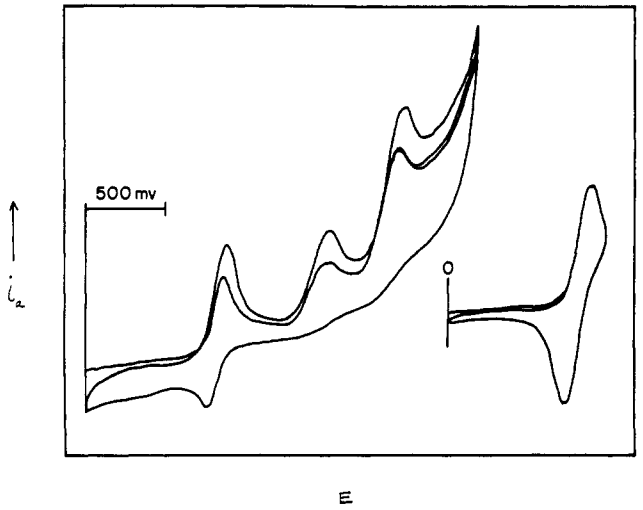


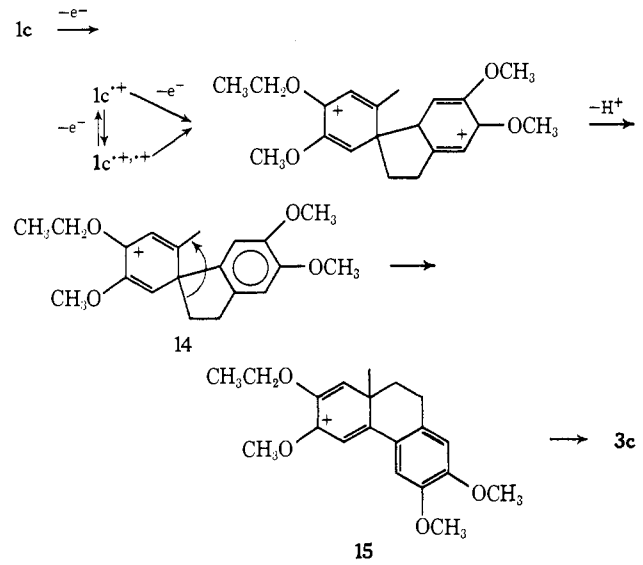
Figure 1. Cyclic voltammogram of 4,5-dimethylveratrole; scan rate 500 mV/sec.

Table I. Peak Potentials from Cyclic Voltammetry[a]

Substrate	E_{P_a}, V
2-Methyl-4,5-dimethoxybibenzyl (**1d**)	0.87, 1.44, and 1.87
2-Methyl-3′,4′,4,5-tetramethoxybibenzyl (**1a**)	0.90, 1.17, 1.37, and 1.65
2-Methyl-2′,4,5′,5-tetramethoxybibenzyl (**1b**)	0.90, 1.00, and 1.40
4,5-Dimethylveratrole	0.90, 1.57, and 1.80
4-Methylveratrole	0.97, 1.12, 1.57, and 2.20
9,10-Dihydro-10a-methyl-2,6,7-trimethoxy-3(10a*H*)-phenanthrone (**3a**)	1.10, 1.3, 1.8

[a] CH_3CN–$LiClO_4$ solution, Ag|0.1 *N* $AgNO_3$ reference, scan rate 500 mV/sec over 0–2-V range.

Scheme II



for **1c**. It is first noted that compound **1d** gave a somewhat more stable cation radical (cyclic voltammetry) and did not cyclize to an appreciable extent. This is rationalized by considering the unreactivity of phenyl compared to dialkoxyphenyl. If an initially formed cation radical (**1c**$^{\cdot+}$) attacked an unoxidized ring, it would resemble an electrophilic attack and the phenyl would be relatively inert. Alternatively, it may be that

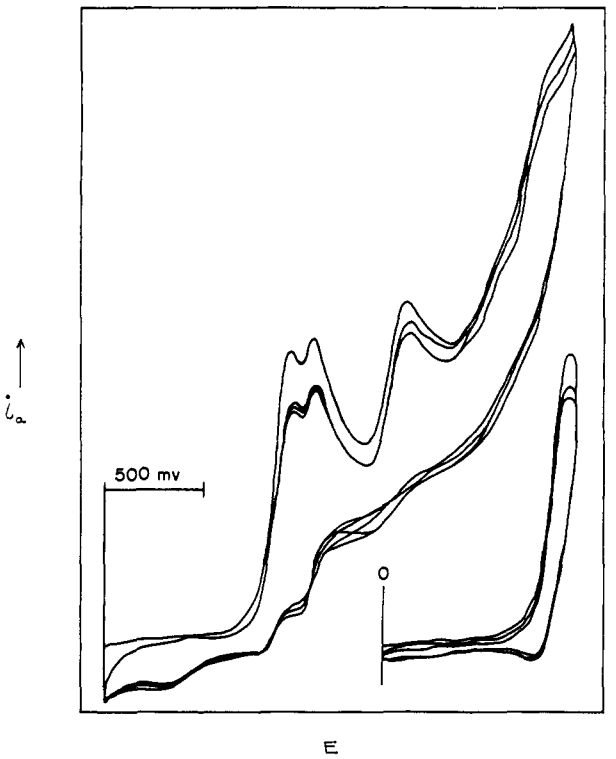


Figure 2. Cyclic voltammogram of 2-methyl-3′,4,4′,5-tetramethoxybibenzyl; scan rate 500 mV/sec.

both rings are being oxidized to produce a bis-cation radical ($1c^{+\cdot,+\cdot}$) which gives radical coupling.[4b] Again phenyl would be unreactive due to its relatively high oxidation potential compared to the dialkoxy phenyl ring.

We assume, therefore, for **1a–c**, that coupling is the rapid follow-up reaction after initial one- or two-electron transfer. After rearomatization of one ring by deprotonation, one can reach the cation **14**. This cation by migration of the ethylene bridge gives a new, more stable cation, **15**, which finally leads to the product by loss of methyl.

A major product in the anolyte is the red material (λ_{max} 416,503 nm) which we suggest is **15**. The same species can be produced by independently treating **3c** with trimethyloxonium fluoroborate. The hexadienone is thus methylated giving back the relatively stable cation. Since independently produced **15** is also hydrolyzed back to **3c**, this strongly implies that demethylation is the last step in the reaction.

Two points are of special interest in this proposed mechanism because of their novelty. The first is the formation of the five-membered ring *via* coupling instead of a six-membered ring. Since the *O*-methyl group is lost in the oxidation of **1c**, it is indicated that the ring methyl does not migrate but rather that the bridge undergoes a carbonium ion rearrangement of the type common to dienone–phenol rearrangements. The extra stability imparted by conjugation of the cation with the dimethoxybenzene ring in **15** clearly explains why this is the ultimate product.

The reactivity of **1d** has literature analogies. Thus, alkylbenzenes oxidize under these conditions to yield the corresponding benzyl alcohol, ketone, and acetamide.[10] Cleavage of benzylic bonds is also common in cases where stable cation fragments can be generated.[11] In the case of **1d**, benzyl cations are sufficiently stable to allow some cleavage to yield **9**, **10**, and **11**.

Experimental Section

Products and starting material were analyzed by ir on a Perkin-Elmer 457 grating spectrometer. Nmr spectra were taken on a Varian A-60A spectrometer, and chemical shifts are reported in δ. Mass spectra were measured with an A.E.I. Model MS-12 spectrometer, and uv-visible with a Cary Model 17. Elemental analyses were performed by Chemalytics, Inc., Tempe, Ariz.

General Electrolysis Procedure. Preparative oxidations were conducted in a three-compartment cell (which separated the anode, cathode, and reference electrode solutions by glass frits) in conjunction with a Wenking Model 70 HV1/90 potentiostat. The anode was a stationary platinum sheet (total area 6.25 cm^2). The anode compartment has an approximate 150-ml volume in which solutions were agitated by means of a magnetic stir bar. A stainless steel sheet served as the cathode. A 0.1 *N* $AgNO_3$ solution in acetonitrile in contact with an Ag wire served as the reference; all potentials are given *vs.* this reference. The entire cell was maintained at ice-bath temperature and under a nitrogen atmosphere.

G. F. Smith anhydrous lithium perchlorate was used as background electrolyte. Sufficient electrolyte was added to the anode and cathode compartments to have an approximately 0.1 *M* solution in electrolyte. For preparative oxidations, solid anhydrous Baker AR sodium carbonate was sometimes added to the anode compartment prior to electrolysis but had no discernible effect. Electrolyte and carbonate were used as received and were not truly anhydrous.

A general description of the preparative oxidation is as follows. Approximately 10^{-3} mol of substrate was added to the cell filled with purified acetonitrile. The potential was maintained at 0.85–0.90 V, with initial currents generally 100–80 mA. The background in these potential ranges was less than 1 mA. Electrolysis was usually discontinued when the current dropped to ~5 mA, which generally took 1–1.5 hr. The anolyte was then removed and stripped under vacuum to near dryness (CAUTION, contains perchoric acid, do not take to dryness!) and then taken up in water, and extracted with chloroform. The combined organic layers were dried with anhydrous sodium carbonate, filtered, evaporated, and then crystallized from ethanol.

Cyclic voltammetry was performed at room temperature under a nitrogen atmosphere using a PAR Electrochemistry System Model 170 in a two-compartment cell separating the 0.1 *N* $AgNO_3|Ag$ reference solution from the platinum anode button and cathode button by a glass frit. In the latter, the total service volume of the working compartment is approximately 15 ml. Voltammograms were recorded in both the presence and absence of solid sodium carbonate with no detectable variation. Either lithium perchlorate or tetramethylammonium tetrafluoroborate was used to maintain an approximate 0.1 *N* solution of electrolyte.

All cyclic voltammetric studies and preparative electrolyses were conducted in Kodak <0.1% water grade acetonitrile, which had been distilled twice from P_2O_5 with nitrogen bleed and stored over molecular sieves under inert atmosphere until used.

3,4-Dimethoxytoluene. According to the method of Bruce and Sutcliffe,[5] a mixture of veratraldehyde (91.5 g), KOH (100 g), 95% hydrazine (75 ml), and ethylene glycol (700 ml) was heated at reflux until the KOH disappeared (30 min). The hydrazone separated as a yellow solid and mild foaming occurred. A continuation of the heating at reflux caused evolution of N_2 and vigorous frothing. After 3 hr, the solution was cooled and poured into 1.5 l. of cold water, and the resulting oil was extracted into ether. The combined extracts were washed with water, dried, and evaporated to yield a yellow oil which was distilled to give 65.7 g of the product as a colorless oil (bp 68–72° (0.05 mm)) whose nmr was identical with that published.[9]

4,5-Dimethoxy-2-(3,4-dimethoxyphenylacetyl)toluene (2a). To a stirring solution of 35.6 g (0.24 mol) of 3,4-dimethoxytoluene and 14.6 g (0.11 mol) of $AlCl_3$ in 200 ml of CS_2 was added dropwise over the course of 1 hr 0.1 mol of 3,4-dimethoxyphenylacetyl chloride

(10) L. L. Miller and E. A. Mayeda, *Tetrahedron*, **28**, 3375 (1972); L. Eberson and K. Nyberg, *Accounts Chem. Res.*, **6**, 106 (1973).

(11) L. L. Miller, V. R. Koch, M. E. Larscheid, and J. F. Wolf, *Tetrahedron Lett.*, 1389 (1971); J. M. Bobbitt, K. H. Weisgraber, A. J. Steinfeld, and S. G. Weiss, *J. Org. Chem.*, **35**, 2884 (1970).

(freshly prepared from 3,4-dimethoxyphenylacetic acid). The mixture was stirred overnight at 25° and poured into ice and the resulting oil extracted into benzene. The extracts were dried and evaporated to yield a black viscous oil from which excess dimethoxytoluene was distilled *in vacuo*. After the pot residue had cooled, alcohol was added and the mixture was allowed to stand 24 hr at 0°. This allowed isolation of essentially pure ketone **2a** as a brown powder (25.4 g or 80% yield based upon the acid chloride). An analytical sample was recrystallized from EtOH–EtOAc to yield **2a** as white needles: mp 115°; nmr (δ) 2.52 (s, 3 H), 3.82 (s, 6 H), 3.85 (s, 3 H), 3.88 (s, 3 H), 4.15 (s, 2 H), 6.68 (s, 1 H), 6.77 (s, 3 H), 7.30 (s, 1 H).

Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.84; H, 6.93.

2-Methyl-3′,4,4′,5-tetramethoxybibenzyl (1a). Reaction of 8.0 g of **2a** and 10 g of KOH in a solution of 6.5 ml of 95% hydrazine in 84 ml of triethylene glycol according to the above procedures gave a yellow oil which was crystallized from alcohol to give 6.4 g (82%) of **1a** as a white powder: mp 101.5–102.5°; nmr (δ) 2.20 (s, 3 H), 2.82 (s, 4 H), 3.80 (s, 3 H), 3.84 (s, 9 H), 6.58–6.80 (m, 5 H); mass spectrum *m/e* (rel intensity) 316 (5), 165 (100), 151 (15).

Anal. Calcd for $C_{19}H_{24}O_4$: C, 72.13; H, 7.65. Found: C, 72.15; H, 7.69.

9,10-Dihydro-10a-methyl-2,6,7-trimethoxy-3(10a*H*)-phenanthrone (3a). Oxidation of 316 mg of **1a** at 0.90 V at 0° gave a dark brown solid which was recrystallized from alcohol and combined with crystals from the mother liquors to yield 295 mg (98%) of **3a** as buff colored needles, mp 204–205°. An analytical sample was recrystallized from ethanol to give white needles: mp 205–206°; nmr (δ) 1.28 (s, 3 H), 1.70–2.10 (m, 2 H), 2.82–3.15 (m, 2 H), 3.78 (s, 3 H), 3.95 (s, 6 H), 5.82 (s, 1 H), 6.63 (s, 1 H), 6.70 (s, 1 H), 7.13 (s, 1 H); ir λ_{max} (Nujol) 1640, 1625, 1605, 1575 cm^{-1}; mass spectrum *m/e* (rel intensity) 300 (17), 299 (63), 257 (100); uv (EtOH) λ_{max} (log ε) 212 (4.74), 238 (4.33), 265 (4.35), 292 (4.26), 357 (4.41).

Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.96; H, 6.65.

4,5-Dimethoxy-2-(2,5-dimethoxyphenylacetyl)toluene. Reaction of 74.1 g of 3,4-dimethoxytoluene and 18.6 g of $AlCl_3$ in 300 ml of CS_2 with 25 g of freshly prepared 2,5-dimethoxyphenylacetyl chloride gave 30.5 g of **2b** as a white powder, mp 76–77°, from ethanol: nmr δ 2.53 (s, 3 H), 3.78 (s, 6 H), 3.90 (s, 3 H), 3.93 (s, 3 H), 4.16 (s, 2 H), 6.70–6.88 (m, 4 H), 7.40 (s, 1 H).

Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71. Found: C, 68.95; H, 6.52.

2-Methyl-2′,4,5′,5-tetramethoxybibenzyl (1b). Reaction of 16.5 g of 4,5-dimethoxy-2-(2,5-dimethylphenylacetyl)toluene in 200 ml of diethylene glycol with 20 g of KOH and 12 ml of 95% hydrazine according to the procedure for preparation of **1a** yielded a yellow oil which crystallized from alcohol to give 14.3 g (88%) of **1b** as a light brown powder, mp 69–70°. An analytical sample was prepared by sublimation to yield a white powder: mp 71–72°; nmr δ 2.25 (s, 3 H), 2.83 (s, 4 H), 3.66 (s, 3 H), 3.70 (s, 3 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 6.62–6.80 (m, 5 H).

Anal. Calcd for $C_{19}H_{24}O_4$: C, 72.13; H, 7.65. Found: C, 71.99; H, 7.73.

9,10-Dihydro-10a-methyl-2,5,8-trimethoxy-3(10a*H*)-phenanthrone (3b). Oxidation of 343 mg of **1b** at 0.86 V gave a dark colored solid after the usual work-up. Crystallization of the solid and work-up of the mother liquors in the same way gave 288 mg (88%) of **3b**: mp 184–185.5°; nmr δ 1.26 (s, 3 H), 1.75–2.08 (m, 2 H), 2.68–3.02 (m, 2 H), 3.74 (s, 3 H), 3.78 (s, 3 H), 3.81 (s, 3 H), 5.82 (s, 1 H), 6.77 (s, 2 H), 7.23 (s, 1 H); ir (Nujol) 1650, 1630, 1600, and 1580 cm^{-1}; uv (EtOH) λ_{max} (log ε) 213 (4.55), 235 sh (3.99), 273 (4.22), 286 (4.27), 315 sh (4.03), 357 (3.85).

Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.63; H, 6.63.

3-Ethoxy-4-methoxybenzaldehyde. To a solution of 100 g of 3-hydroxy-4-methoxybenzaldehyde in 200 ml of ethanol was added a solution of 44 g of KOH in 44 ml of H_2O. The mixture dissolved upon heating to reflux and 56 ml of ethyl bromide was then added slowly. A yellow solid began to precipitate after 15 min and heating of the mixture at reflux was continued overnight. Excess ethyl bromide and solvent were removed *in vacuo* and the residue was distributed between water and $CHCl_3$. The $CHCl_3$ layer was dried and evaporated to give a pale yellow oil which crystallized from ethanol to give 112 g (93%) of 3-ethoxy-4-methoxybenzaldehyde, mp 49.5–50.5° (lit.[12] mp 50–51°).

3-Ethoxy-4-methoxytoluene. 3-Ethoxy-4-methoxybenzaldehyde (112 g) was treated with 120 g of KOH, 87 ml of 95% hydrazine, and 840 ml of diethylene glycol according to the above procedure to yield a yellow oil. Distillation yielded 94 g (91%), bp 64–69° (1 mm) (lit.[13] bp 120° (30 mm)).

5-Ethoxy-4-methoxy-2-(3,4-dimethoxyacetyl)toluene. 3-Ethoxy-4-methoxytoluene (94 g) was reacted with 74.8 g of $AlCl_3$ and 29.4 g of 3,4-dimethoxyphenylacetyl chloride in 300 ml of CS_2. A total of 40.0 g (87%) of **2c** was obtained after crystallization from ethanol: mp 106–107°; nmr δ 1.47 (t, 3 H, J = 7 Hz), 2.52 (s, 3 H), 3.88 (s, 9 H), 4.15 (q, 2 H, J = 7 Hz), 4.16 (s, 2 H), 6.75 (s, 1 H), 6.80 (s, 3 H), 7.35 (s, 1 H).

Anal. Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.42; H, 7.23.

2-Methyl-3′,4′,5-trimethoxy-4-ethoxybibenzyl (1c). By the procedure for the preparation of **1a**, 35.1 g of the ketone was reacted with 20 g of KOH, 14.5 ml of 95% hydrazine, and 140 ml of diethylene glycol to give 25 g of **1c** as a colorless oil (bp 175° (0.1 mm)). The oil could be obtained crystalline from ethanol with care: mp 38–40°; nmr δ 1.42 (t, 3 H, J = 7 Hz), 2.19 (s, 3 H), 2.79 (s, 4 H), 3.76 (s, 3 H), 3.79 (s, 3 H), 3.81 (s, 3 H), 4.07 (q, 2 H, J = 7 Hz), 6.58–6.79 (m, 5 H).

Anal. Calcd for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.68; H, 8.16.

9,10-Dihydro-10a-methyl-2-ethoxy-6,7-dimethoxy-3(10a*H*)-phenanthrone (3c). Oxidation of 360 mg of **1c** at 0.80 V yielded 340 mg of black oil after the usual work-up. This oil was recrystallized from ethanol to yield 302 mg (86%) of **3c**; mp 178–179.5°; nmr δ 1.21 (s, 3 H), 1.45 (t, 3 H, J = 7 Hz), 1.95 (m, 2 H), 2.95 (m, 2 H), 3.80 (q, 2 H, J = 7 Hz), 3.90 (s, 9 H), 5.79 (s, 1 H), 6.60 (s, 1 H), 6.68 (s, 1 H), 7.08 (s, 1 H); uv (ethanol) λ_{max} 238 (log ε = 3.99), 264 (log ε = 4.01), 289 (log ε = 3.93), 353 (log ε = 4.11).

Anal. Calcd for $C_{19}H_{22}O_4$: C, 72.60; H, 7.01. Found: C, 71.8; H, 6.9.

6-Methylveratraldehyde. Closely following literature methods,[5] freshly distilled $POCl_3$ (50.6 ml) was added dropwise during 30 min to a stirred mixture of 4-methylveratrole (65.7 g) and DMF (30 g) freshly distilled after standing 2 days over $CuSO_4$. Heating overnight on a steam bath under reflux gave a deep brown, viscous solution which when cooled was admixed with 600 ml of water. Addition of excess 10% NaOH solution separated out a yellow oil which was extracted with benzene. The combined extracts were washed with water, dried, and evaporated to give a viscous yellow oil. Crystallization from alcohol gave 39.4 g of the aldehyde as a white powder: mp 74–75° (lit.[5] 74°); nmr δ 2.58 (s, 3 H), 3.90 (s, 3 H), 3.95 (s, 3 H), 6.68 (s, 1 H), 7.30 (s, 1 H), 10.20 (s, 1 H).

4,5-Dimethylveratrole. 6-Methylveratraldehyde (1.80 g) was treated with 2 g of KOH, 1.4 ml of 95% hydrazine, and 20 ml of diethylene glycol in the manner described for the preparation of 3,4-dimethoxytoluene to give a quantitative yield of 4,5-dimethylveratrole as a cream colored solid. An analytical sample was sublimed (60° (0.05 mm) to give a white powder; mp 41–42° (lit.[5] 42.5°); nmr δ 2.19 (s, 6 H), 3.85 (s, 6 H), 6.66 (s, 2 H).

α-Hydroxy-4,5-dimethoxy-2-methylbibenzyl. To 10 g of 6-methylveratraldehyde under a nitrogen atmosphere in 75 ml of dry THF was added dropwise 1.5 equiv of benzylmagnesium chloride in 20 ml of dry THF. After stirring overnight at room temperature, 20 ml of water was added. The residue remaining after most of the THF had been distilled off was added to an additional 100 ml of water and extracted with ether to give a yellow oil. Distillation under reduced pressure gave bibenzyl resulting from coupling of the Grignard reagent and a quantitative yield of the adduct **12** as a colorless viscous oil: bp 170–175° (0.05 mm); nmr δ 2.08 (s, 3 H), 2.88 (distorted doublet, J = 6 Hz, integration for 3 H suggests coincidence with the alcohol proton), 3.75 (s, 6 H), 4.96 (t, J = 6 Hz, 1 H), 6.52 (s, 1 H), 7.00 (s, 1 H), 7.15 (s, 5 H).

Anal. Calcd for $C_{17}H_{20}O_3$: C, 74.97; H, 7.40. Found: C, 75.38; H, 7.43.

4,5-Dimethoxy-2-methylbibenzyl (1d). To a solution of 10 g of **12** in 200 ml of dry ether and 50 ml of dry THF containing 0.5 ml of pyridine was added portionwise a solution of 10 ml of thionyl chloride in 20 ml of dry ether during 30 min with stirring. After an additional 20 min at room temperature, the reaction was cautiously quenched with cold water. Separation and evaporation of the ethereal layer gave 10 g of product as a pale yellow, mobile oil. The ir showed no OH absorption. A solution of 1 g of this product in 5 ml of dry ether was added dropwise to a stirred solution of 300

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(13) A. A. Goldberg and H. S. Turner, *J. Chem. Soc.*, 111 (1946).

mg of LiAlH in 50 ml of dry ether at room temperature. After stirring overnight, the reaction was cautiously quenched with wet ether. The separated aluminate was filtered and washed generously with ether. The organic filtrate was washed with water, dried, and evaporated to give a mobile yellow oil. Distillation (110–115° (0.05 mm)) gave 680 mg of colorless oil: nmr δ 2.20 (s, 3 H), 2.80 (s, 4 H), 3.75 (s, 3 H), 3.80 (s, 3 H), 6.53 (s, 1 H), 6.60 (s, 1 H), 7.17 (s, 5 H); mass spectrum *m/e* 256 (13), 165 (100), and 91 (39).

Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.41; H, 8.11.

Somatostatin. Total Solid Phase Synthesis

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Abstract: The structure of a hypothalamic somatotropin-release inhibiting factor (SRIF, somatostatin) of ovine origin, the sequence of which is H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH, has been reported. We describe: (a) the synthesis on a chloromethylated resin of the corresponding linear peptide; (b) its deprotection and cleavage from the resin by HF; (c) its purification by repeated gel filtrations in presence of β-mercaptoethanol; (d) oxidation of the sulfhydryls of both cysteinyl residues to form the 38-membered ring; (e) characterization of the straight-chain intermediate and bridged tetradecapeptide after purification; (f) comparison of natural somatostatin with its synthetic counterpart by tlc in three different systems, electrophoresis on paper, amino acid analysis after hydrolysis under various conditions, optical rotation, circular dichroism, gel filtration and partition chromatography on Sephadex G-25F, tlc of the tryptic digest, mass spectrometry of the derivatized fragment -Asn-Phe-Phe-Trp-Lys- isolated from a tryptic digest, and biological activity.

The tetradecapeptide somatostatin,[1] H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH, was isolated from ovine hypothalamic extracts[2] on the basis of its ability to inhibit the secretion of radioimmunoassayable growth hormone (GH) by primary cultures of enzymatically dispersed rat anterior pituitary cells. The sequence was obtained using the regular Edman degradation procedure[3] coupled to mass spectrometric analysis of a tryptic digest.[4] Extensive biological studies made on a synthetic material, the synthesis of which has already been described in a preliminary note,[5a] have been reported.[6–8] It is noteworthy that somatostatin has been found to inhibit in normal human subjects the release of growth hormone brought about by L-Dopa, infusion of arginine,[9,10] and in diabetics, by exercise;[11] it is highly active in lowering plasma levels of GH in acromegalics[10] and thus, as originally proposed,[1] may be of therapeutic value in acromegaly and diabetes. We report here in detail the first synthesis[5a] of somatostatin in a highly purified form and results from the comparison of the physical and biological properties of both synthetic and natural peptides. This comparison should not be considered as a proof of reported structure[1,3,4] by synthesis but as another means of identification of the synthetic material made by the still controversial solid phase methodology, the drawbacks and advantages of which are discussed for this particular peptide.

The protected somatostatin tetradecapeptide was synthesized in a stepwise manner on chloromethylated resin prepared according to Stewart and Young.[12] Boc-Cys(*p*-OMe-Bzl) was esterified by the classical method,[12] even though esterification in DMSO in the presence of potassium *tert*-butoxide according to Monahan and Gilon[13] was also found to be appropriate. For both methods the optical purity of the cysteine attached to the resin was checked by the synthesis of the dipeptide Leu-Cys(Cm). Boc-Leu was coupled to the free Cys(*p*-OMe-Bzl)-polymer: the dipeptide was deprotected and cleaved from the resin in HF[14] and

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