Anal. Calcd for $C_{26}H_{26}INO_4$: C, 57.5; H, 4.8. Found: C, 57.3; H, 4.6.

(±)-**Cryptowoline Iodide (XIII).**—A solution of 1.3 g of XII in 100 ml of methanol was shaken with hydrogen (50 psi) at 25° for 3 hr in the presence of 0.2 g of 10% palladium on charcoal. After removal of the catalyst by filtration and evaporation of the solvent, 1.25 g (95%) of the starting material was recovered unchanged. Therefore, to effect debenzylation of XII, a 200-mg sample dissolved in a mixture of 5 ml of methanol and 3 ml of hydrochloric acid was heated on a steam bath for 10 min and then evaporated to dryness under reduced pressure at 45°. The residue crystallized when triturated with methanol to yield 100 mg (60%) of XIII, mp 150–151° dec.

Anal. Calcd for $C_{19}H_{20}INO_4$: C, 50.4; H, 4.4. Found: C, 50.3; H, 4.4.

Registry No.—XIII, 7695-55-8; 3-methoxy-4-benzyloxyphenylacetonitrile, 1700-29-4; VII, 7687-04-9; VII, 7686-98-8; IX, 7686-99-9; (+)-1-(2′-bromo-4′,5′-methylenedioxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline, 7690-89-3; (−) base, 7687-00-5; XI, 7687-01-6; X, 7687-02-7; XII, 7687-03-8.

Acknowledgment.—This research was supported by a Battelle Memorial Institute grant. We are indebted to R. J. Jakobsen and T. F. Page for the measurement of infrared spectra. We also wish to thank Dr. E. Richie for a reference sample of natural (−)-cryptowoline iodide.

A Practical Synthesis of Protostephanine

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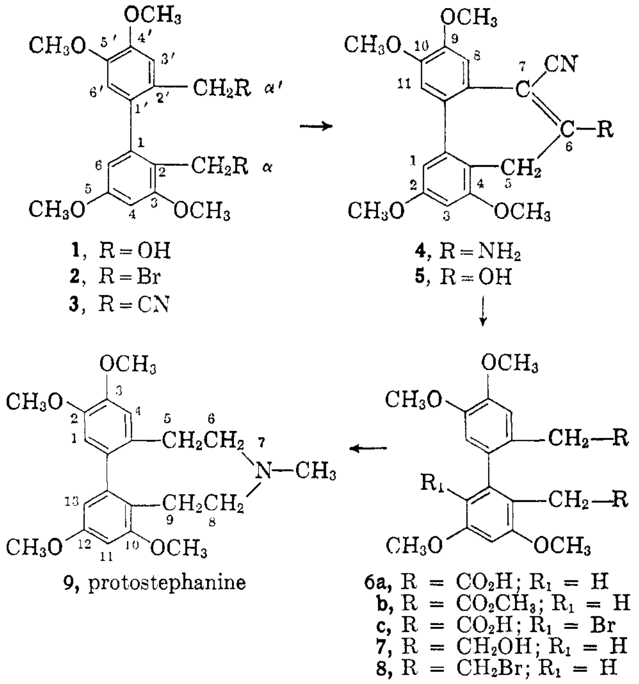
A synthesis of the alkaloid protostephanine, 6,7,8,9-tetrahydro-2,3,10,12-tetramethoxy-7-methyl-5H-dibenz[*d,f*]azonine, is described. The readily available 3,4′,5,5′-tetramethoxy-2,2′-biphenyldimethanol is converted into its homologous dibromide which is treated with methylamine to yield protostephanine identical with the natural material. The yield obtained in the ring-closure stage (34%) indicates that the formation of dibenz[*d,f*]azonines in this manner occurs more readily than might have been anticipated.

The structure determination of the alkaloid protostephanine, isolated from *Stephania japonica*, Miers, was reported in a series of papers by Kondo, Takeda, *et al.*,[1] to be 6,7,8,9-tetrahydro-2,3,10,12-tetramethoxy-7-methyl-5H-dibenz[*d,f*]azonine (**9**). Recently Takeda[2] gave added support for this formula by nmr spectra and a synthesis. However, the synthesis produced insufficient material for a satisfactory comparison with the natural product.[3]

In this paper a practical synthesis of protostephanine is described which starts from the readily available diol (**1**).[4] This approach resembles that of Takeda; however, it differs in many important details and proceeds as shown in Scheme I.

Diol **1** was first converted to diacetonitrile **3** *via* dibromide **2**. Attempts to hydrolyze **3** to the corresponding diacetic acid (**6a**) with acidic or basic reagents were unsuccessful. The following sequence gave **6a** in high yield. Treatment of diacetonitrile **3** in ethanol with a catalytic amount of sodium ethoxide gave a cyclic aminonitrile, probably **4**.[5] Acid hydrolysis of **4** yielded the hydroxynitrile (**5**). Treatment of the latter compound with methanolic sodium hydroxide caused simultaneous saponification of the cyano group and ring cleavage yielding the desired diacetic acid (**6a**) in an over-all yield of 84% based on the original diacetonitrile.

Scheme I



The detailed structures of compounds **4** and **5** are of interest. In the solid state, compound **4** exists entirely as the aminonitrile as indicated by the infrared spectrum. There was no evidence for the presence of corresponding tautomer **4a** (Chart I). However, the product obtained by acid hydrolysis of **4** seems to be a mixture of hydroxynitrile **5** and ketonitrile **5a** (Chart I), since the infrared spectrum shows a hydroxyl band at 3585, and carbonyl band at 1730 cm^{-1} and two cyano bands. One of these at 2250 cm^{-1} corresponds to the unconjugated ketonitrile while the other at 2200 cm^{-1} is attributed to a conjugated cyano group.

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[1] For a summary and review of the early work, *cf.* H.-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960," Akademie Verlag, Berlin, 1961, p 402, and the references given in our previous paper: B. Pecherer and A. Brossi, *Helv. Chim. Acta*, **49**, 2261 (1966).

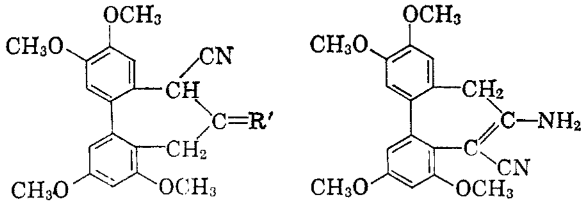
[2] K. Takeda, *Itsuu Kenkyusho Nempo*, **13**, 45 (1963); *Chem. Abstr.*, **60**, 5570*f* (1964).

[3] The comparison of the synthetic and natural materials depended on the similarity of the R_f value on paper strip chromatography and a micro melting point determination. While the R_f values for both materials were identical, the melting point of the synthetic material was 65–68° whereas the natural alkaloid melts at 70–75°. These are data for the methanol complex of the alkaloid, but our experience has shown that the melting points do not constitute a reliable criterion in this series.

[4] Diol **1** is compound IX of the previous paper.[1]

[5] The present data, however, do not exclude structure **4c** (Chart I) which would originate by an alternate mode of ring closure. In this latter case the cyano group would occupy position 5.

CHART I



4a, R′ = NH
5a, R′ = O

4c

Diacetic acid **6a** was further characterized as its methyl ester (**6b**). Hydride reduction of either **6a** or **6b** gave diol **7**, a compound previously obtained by Takeda[2] by reduction of the dimethyl ester of 6-bromo-3,4′,5,5′-tetramethoxy-2,2′-biphenyldiacetic acid (**6c**) with lithium aluminum hydride. Treatment of diol **7** with phosphorus tribromide in ether gave the corresponding dibromo compound (**8**), a stable, crystalline substance melting at 91–92°.[6]

Treatment of dibromo compound **8** with excess methylamine in benzene solution at 130–150° for 2 hr gave a mixture of bases from which after chromatography and crystallization, a tertiary amine melting at 84–86° was isolated. This substance was identical with natural protostephanine,[7] on the basis of microanalysis, nmr, infrared, mass spectrum, and thin layer chromatography.

It now seems evident that the formation of nine-membered rings of the dibenz[*d,f*]azonine series is not as difficult as might have been anticipated,[8,9] since a 34% yield of protostephanine was obtained. It appears that the poor yield reported by Takeda[2] was probably due to the large proportion of by-products present in the mixture resulting from the bromination of the diol **7**.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are corrected. Infrared spectra were determined on a Beckman Model IR-5 recording spectrophotometer. Nmr spectra were determined on a Varian A-60 spectrometer with tetramethylsilane as the internal standard. Mass spectra were determined on a CEC 21-110 instrument.

α,α′-Dibromo-3,4′,5,5′-tetramethoxy-*o,o*′-bitolyl (2).—To a stirred suspension of 70 g (0.21 mole) of 3,4′,5,5′-tetramethoxy-2,2′-biphenyldimethanol (1) in 2.1 l. of dry ether, 56.8 g (0.21 mole) of phosphorus tribromide was added dropwise at −4 to 0° over a 20-min period, and then the mixture was allowed to warm to room temperature. After 24 hr of stirring, a dense, white solid had precipitated. The suspension was poured into ice water, 1 l. of benzene was added, and the mixture was stirred until all the solid had dissolved. The organic phase was washed successively with water, saturated bicarbonate solution, and water and dried. Removal of the solvent at reduced pressure yielded 92.8 g (96%) of a buff solid, mp 118–120°. After recrystallization from petroleum ether (bp 60–90°) white crystals (mp 124–126°) were obtained. Infrared ($CHCl_3$) showed 3000, 2940, 2830, 1603, and 1520 cm^{-1}.

Anal. Calcd for $C_{18}H_{20}Br_2O_4$: C, 46.98; H, 4.38; Br, 34.73. Found: C, 46.89; H, 4.63; Br, 34.84.

3,4′,5,5′-Tetramethoxy-2,2′-biphenyldiacetonitrile (3).—One hundred and seventy grams (0.37 mole) of the dibromo compound (2) was added to a stirred suspension of 76 g (1.11 moles) of potassium cyanide (95%) in 3 l. of dimethyl sulfoxide. The temperature rose to 30° and after 45 min at this temperature, the mixture was poured into ice water with vigorous stirring, whereupon the initially formed oil solidified. The solid was collected by filtration, washed with water, and dried giving 104 g (78%) of (3), mp 105–107°. Recrystallization from ethanol gave 69 g (52%) of tan crystals, mp 109–111°. A colorless analytical sample was obtained by one more recrystallization from ethyl acetate–petroleum ether (bp 60–90°): mp 116.5–118.5°; infrared ($CHCl_3$) at 3005, 2960, 2930, 2840, 2245, 1600, 1520 cm^{-1}; nmr (DMSO-d_6) at δ 3.14 and 3.56 (J = 17 cps, CH_2), 3.60 (CH_2), 3.80, 3.83, 3.88, and 3.95 (4 CH_3O), and 6.48, 6.75, 6.82, and 7.20 (4 aromatic).

Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 67.92; H, 5.98; N, 8.04.

6-Amino-2,4,9,10-tetramethoxy-5H-dibenzo[*a,c*]cycloheptene-7-carbonitrile (4).—Sixty-four grams of **3** was dissolved in 700 ml of boiling ethanol and 10.9 ml of a 3% solution of sodium ethoxide was added. After 2 hr of refluxing, the solution was chilled for 18 hr. The crystalline solid which separated was collected, washed with ethanol, and dried to yield 64 g of white solid: mp 197–198°;[10] infrared (KBr) at 3450, 3350, 3220, 2930, 2828, 2190, 1635, 1590, and 1515 cm^{-1}.

Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.23; H, 6.02; N, 7.82.

6-Hydroxy-2,4,9,10-tetramethoxy-5H-dibenzo[*a,c*]cycloheptene-7-carbonitrile (5) ⇄ 6,7-Dihydro-2,4,9,10-tetramethoxy-6-oxo-5H-dibenzo[*a,c*]cycloheptene-7-carbonitrile (5a).—Sixty-four grams of **4** was refluxed for 0.5 hr with 1.82 l. of 6 *N* hydrochloric acid and a few milliliters of xylene. The suspension was chilled for 1 hr, and the solid was collected, washed free of acid, and dried. This product (64 g, mp 216–218°) after recrystallization from ethanol, melted at 220–221.5°. The infrared (KBr) showed a sharp band at 3585 and others at 3010, 2960, 2940, 2250, 2200, 1740, 1610, and 1510 cm^{-1}, which indicated that in the solid state the substance was a keto–enol mixture of **5** and **5a**. As in the case of **4**, the exact position (5 or 7) of the cyano group in structure **5** is uncertain.[5]

Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 67.66; H, 5.59; N, 3.86.

3,4′,5,5′-Tetramethoxy-2,2′-biphenyldiacetic Acid (6a).—A suspension of 64 g (0.181 mole) of **5** in a mixture of 640 ml of 30% sodium hydroxide and 400 ml of methanol was refluxed for 24 hr. The cooled, turbid solution was diluted with 2 l. of water and extracted with two 300-ml portions of ether. Acidification of the aqueous layer precipitated an oil which was extracted with three 300-ml portions of chloroform. The combined extracts were washed with water and dried. Distillation of the solvent under reduced pressure gave an oily residue which solidified when triturated with ethyl acetate. Filtration gave 59.4 g (84%) of a white solid, mp 193.5–194.5°, which after recrystallization from ethyl acetate melted at 197–198°; infrared (KBr) at 2950, 1710, 1605, 1590, and 1515 cm^{-1}; nmr (DMSO-d_6) at δ 3.03 and 3.27 (J = 17 cps, CH_2), 3.28 (CH_2), 3.70, 3.75, 3.80, and 3.80 (4 CH_3O), and 6.32, 6.58, 6.70, and 6.93 (4 aromatic).

Anal. Calcd for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.84; H, 5.59.

Dimethyl Ester of 3,4′,5,5′-Tetramethoxy-2,2′-biphenyldiacetic Acid (6b).—The acid (6a) was esterified in methanol with an excess of diazomethane. Recrystallized from methanol, the ester melted at 100–101.5°; infrared ($CHCl_3$) at 3025, 2935, 2835, 1730, 1610, 1515 cm^{-1}; nmr ($CDCl_3$) at δ 3.23 and 3.43 (J = 17 cps, CH_2), 3.60, 3.60, 3.78, 3.82, 3.82, and 3.92 (6 CH_3O); and 6.39, 6.52, 6.77, and 6.83 (4 aromatic).

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[6] Takeda[2] reported this substance to be an oil. When prepared by either his or our method, the reaction product contains no more than 25% of the desired substance.

[7] A sample of the natural product was kindly supplied by Professor K. Tsuda of the Institute of Applied Microbiology of the University of Tokyo.

[8] In a recent patent which came to our attention near the completion of our work, similar results were described; *cf.* Japanese Patent Application 5384/66 (Shionogi and Co.), described in Derwent No. 20,990.

[9] The cyclization of diamides of **6a**, in the manner described for *o*-phenylenediacetamide by J. O. Halford and B. Weissmann [*J. Org. Chem.*, **17**, 1646 (1952)] was attempted, but no cyclic imide could be detected. Likewise, cyclization of **3** by hydrogenation in the manner described by P. Ruggli, B. B. Bussemaker, W. Müller, and A. Staub [*Helv. Chim. Acta*, **18**, 1388 (1935)] for *o*-phenylenediacetonitrile was attempted and failed.

[10] In some experiments the product separated as a lower melting form, mp 169.5–171.5, unchanged on recrystallization from ethanol unless seeded with the higher melting form. On tlc both forms showed the same R_f value. The available data seem to indicate that the cyclization product is a single compound; however, whether its structure is **4** or **4c** cannot be established with certainty.

Anal. Calcd for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26. Found: C, 63.18; H, 6.37.

3,4′,5,5′-Tetramethoxy-2,2′-biphenyldiethanol (7).—A solution of 59.4 g (0.152 mole) of the diacetic acid in 610 ml of tetrahydrofuran was added dropwise to a stirred suspension of 22.8 g (0.608 mole) of lithium aluminum hydride in 610 ml of tetrahydrofuran. The mixture was refluxed for 2 hr, decomposed in the usual way with water, and then filtered (Filter Aid). From the filtrate, after removal of the solvent, 20 g of an oil was obtained that was dissolved in ethyl acetate. Chilling yielded 18.7 g (35%) of **7**, mp 127–128°. The cake of oxides was dissolved in 10% hydrochloric acid, the Filter Aid was removed, the filtrate was shaken with chloroform, and the aqueous layer was discarded. The chloroform layer was washed successively with water, saturated bicarbonate solution, and water and dried. Removal of the solvent gave 8 g of oil that on crystallization from ethyl acetate yielded an additional 6.2 g of **7**, mp 127–128°; total yield was 24.9 g (45%). An analytical sample was obtained by recrystallization from ethyl acetate–petroleum ether (bp 60–90°), mp 126–127°;[11] infrared ($CHCl_3$) at 3590, 3500–3300, 3000, 2940, 2820, 2440, 2400, 1600, and 1515 cm^{-1}; nmr ($CDCl_3$) at δ 1.93 (2 OH) 2.64 (2 CH_2), 3.63 (2 CH_2O), 3.78, 3.81, 3.84, and 3.91 (4 CH_3O), and 6.34, 6.50, 6.68, and 6.85 (4 aromatic). The mass spectrum showed a molecular weight of 326.

Anal. Calcd for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.57; H, 7.02.

From the bicarbonate washes starting material was recovered.

2,2′-Bis(2-bromoethyl)-3,4′,5,5′-tetramethoxybiphenyl (8).—One gram (0.00276 mole) of **7** was suspended in 28 ml of dry ether and at 0°, 0.75 g (0.00276 moles) of phosphorus tribromide was added dropwise. After 10–15 min a viscous material separated. Addition of an equal volume of benzene rendered the mixture homogeneous. The mixture was poured into 50 ml of ice water; the organic layer washed with cold water and dried. Distillation of the solvent gave 190 mg of an oil which was dissolved in 3 ml of benzene and the solution was passed through a bed of 5 g of Woelm alumina (grade I). The alumina was washed with 150 ml of benzene and after removal of the solvent from the combined eluates 100 mg of a colorless oil was obtained. The oil solidified, mp 89–91°; recrystallization from petroleum ether (bp 60–90°) raised the melting point to 91–92°; infrared (KBr) at 3000, 2940, 2830, 1605, 1580, and 1515 cm^{-1}; nmr ($CDCl_3$) at δ ~2.90 (2 CH_2), ~3.35 (2 CH_2Br), 3.80, 3.85, 3.87, and 3.95 (4 CH_3O), and 6.32, 6.50, 6.68, and 6.83 (4 aromatic).

Anal. Calcd for $C_{20}H_{24}Br_2O_4$: C, 49.20; H, 4.95; Br, 32.75. Found: C, 48.99; H, 5.01; Br, 32.85.

Protostephanine. 6,7,8,9-Tetrahydro-2,3,10,12-tetramethoxy-7-methyl-5H-dibenz[*d,f*]azonine (9).—Two hundred and fifty milligrams of **8** was heated for 2 hr at 140–150° with a solution of 0.5 g of methylamine in 10 ml of benzene under 200 psi of nitrogen. The product was washed with water. The bases were extracted into 10% hydrochloric acid, liberated with excess potassium hydroxide, and taken up in ether. The ethereal solution was dried and the solvent was distilled to leave 170 mg of a pale-colored syrup which crystallized on standing overnight. The solid was dissolved in 3 ml of benzene and passed over 10 g of Woelm alumina (grade II). Elution with 150 ml of benzene gave 61.8 mg of a white, crystalline solid, mp 84–86°,[12] identical (mass spectrum, nmr, infrared, and tlc) with a natural sample.

Anal. Calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92. Found: C, 70.66; H, 7.84; N, 4.19.

The **sulfate** had mp 130–132° (dried for 4 hr at 100°).

Anal. Calcd for $C_{21}H_{27}NO_4 . H_2SO_4$: C, 55.02; H, 6.74; N, 2.80. Found: C, 55.38; H, 6.42; N, 3.07.

The infrared and nmr spectra of the free base were identical with those reported by Takeda.[2] The high-resolution mass spectrum showed a molecular ion at *m/e* 357.1950 corresponding to the formula $C_{21}H_{27}NO_3$ and in general was very similar to the mass spectrum of natural protestephanine except for some peaks which are attributed to impurities in the natural product. Thin layer chromatography also confirmed the presence of these impurities.

Registry No.—**9**, 549-28-0; **2**, 7732-73-2; **3**, 7732-74-3; **4**, 7732-75-4; **4c**, 7775-85-1; **5**, 7771-31-5; **5** (5-cyano-), 7732-76-5; **5a**, 7732-77-6; **5a** (5-cyano-); 7732-78-7; **6a**, 7732-79-8; **6b**, 7732-80-1; **7**, 10026-45-6; **8**, 7732-81-2.

Acknowledgment.—We wish to thank Mr. M. D. Rosenberg and Mr. R. C. Sunbury for very capable assistance. The microanalyses were performed by Dr. A. Steyermark and his staff. Drs. P. Bommer, F. Vane, and T. Williams, and Mr. S. Traiman provided the mass spectral, nmr, and infrared data.

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[11] Takeda[2] reported mp 123–125°.

[12] Takeda[2] reported mp 65–68°.

The Stucture of Two Isoflavones from the Abyssinian Berebera Tree

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The structures of two isoflavones, apparently earlier isolated by Clark, have been shown to be I and V by spectral and degradative studies.

In the course of his investigations of rotenone-bearing plants, Clark examined the seeds of the Abyssinian Berebera tree, *Millettia ferruginea* (Hochst.) Baker, isolating rotenone and deguelin[1] and artifacts resulting from alkaline treatment and aerial oxidation of the extracts.[2] Alumina chromatography of the filtrates provided two new compounds of mp 164–165° and 189–190°, respectively. Analysis of the lower melting material suggested the formula $C_{21}H_{20}O_6$, with two methoxyl groups, while the higher melting corresponded to $C_{23}H_{20}O_6$, with one methoxyl.

Recently we have been able to examine the seeds of this plant, and have encountered two isoflavones with properties which approximate those of Clark's unknowns.[3] Crystallization of ethyl acetate extracts of these seeds provided a material melting at 182.5–185°, which corresponded in combustion analysis and mass spectrum to $C_{22}H_{18}O_6$. As the nmr spectrum showed a single methoxyl group, it seemed likely that this material was Clark's higher melting compound.[4]

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[1] E. P. Clark, *J. Am. Chem. Soc.*, **65**, 27 (1943).

[2] Clark isolated dehydrorotenone, tephrosin, and isotephrosin, which are not now regarded as natural materials; *cf.* F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworth and Co. (Publishers) Ltd., London, 1963, p 501.

[3] We are indebted to Dr. William Burger of the Chicago Museum of Natural History for the gift of these seeds.

[4] The compositions required by the two different formulas differ by C, 0.57, and H, 0.34; Clark's analyses and ours, fall between the two values, and do not provide a basis for choice. Clark reported a molecular weight determination by an unspecified method which corresponds to the higher formula, but the mass spectrum obviously is a more certain datum. Because Dr. Clark's samples are not available, and no further properties of these materials are reported, the identity of his materials must remain somewhat uncertain.