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Experiments in the Colchicine Field. VI. A Method for the Synthesis of Ring B[1]

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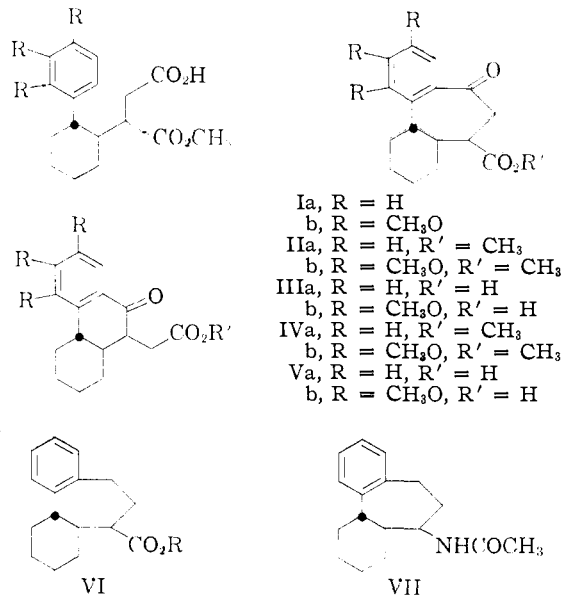
The failure of the trimethoxylated half ester (Ib) to undergo cyclization without ester interchange (*i.e.*, to IIb) prompted the investigation of the usefulness of the ester interchange products (IVa and IVb) as intermediates in the synthesis of tricyclic compounds containing a seven-membered B ring. The conversion of compounds of structure IV to compounds of structure XVII and XVIII has been successfully achieved by reduction of IV to the alcohol VIII or the lactone X, conversion of these to the unsaturated ester XI, ozonolysis and condensation of the resulting intermediate (probably XVI) to XIV or XV (depending upon the method of ozonide decomposition), hydrogenation to XIII or XIX, and hydrolysis with decarboxylation to XVII or XVIII.

The present communication relates to the first paper of this series[3] and describes another method for the construction of ring B in tricyclic compounds of the colchicine type. With this contribution our efforts are brought to a conclusion, for the recent total syntheses of colchicine[4] have put the capstone on research in this area.

Our earlier work[3] described a method for converting the half-ester Ia to the acetylamino compound VII by the sequence of reactions illustrated in the accompanying flow sheet (Ia → IIa → VI → VII). Attempts to extend this method to com-



pounds containing a trimethoxyphenyl A-ring (*i.e.*, starting with Ib) were unsuccessful due to ester interchange during the cyclization reaction of the acid chloride of Ib leading to IVb rather than IIb. The complication of ester interchange had been surmounted in the unmethoxylated series through the use of aluminum bromide as the catalyst.[3] This proved inapplicable in the methoxylated series, however, for conditions which were strenuous enough to induce cyclization also led to demethylation and to the formation of intractable resins. Since milder catalysts gave the ester interchange product IVb in good yield, it was clearly of interest to devise a procedure for converting this type of compound to one containing a seven-membered B ring with a functional group at the appropriate position. To this end the reactions described in the sequential sections were undertaken.

Conversion of IVa to Seven-membered B Ring Compounds.—Reduction of the keto ester IVa[5] or the keto acid Va with aluminum isopropoxide or with sodium borohydride yielded the lactone Xa.[6] On the basis of the known configurations of the starting material, the configuration of the lactone must be *trans* at carbons 3a-3b and carbons 7a-3b; the configuration at carbons 11b-3a, however, is uncertain. The yield of lactone Xa by reduction of keto acid Va did not change significantly upon prolonged heating with aluminum isopropoxide from which one might infer that (a) the product of kinetic control (axial hydroxyl) is trapped *via* irreversible formation of the *cis*-lactone or that (b) the product of thermodynamic control (equatorial hydroxyl) is also that of kinetic control with the result that *trans*-lactone is formed. That the former might be the case is suggested by the carbonyl absorption of Xa. Five-membered lactone rings fused to cyclohexane, cyclohexene and cyclohexadiene rings are reported to have carbonyl absorptions at 1755, 1755 and 1770 cm.$^{-1}$, respectively, for the *cis* isomers and 1780, 1770 and 1781 cm.$^{-1}$, respectively, for the *trans* isomers.[7] Lactone Xa has an absorption at 1761 cm.$^{-1}$, closer to the *cis* series than to the *trans* series.[8] According to

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(2) (a) Universal Match Co. fellow 1958–1960; (b) Postdoctoral fellow 1957–1958; (c) du Pont fellow 1957–1958; (d) Postdoctoral fellow 1954–1955; (e) du Pont fellow 1951–1952.

(3) C. D. Gutsche and K. L. Seligman, *J. Am. Chem. Soc.*, **75**, 2579 (1953).

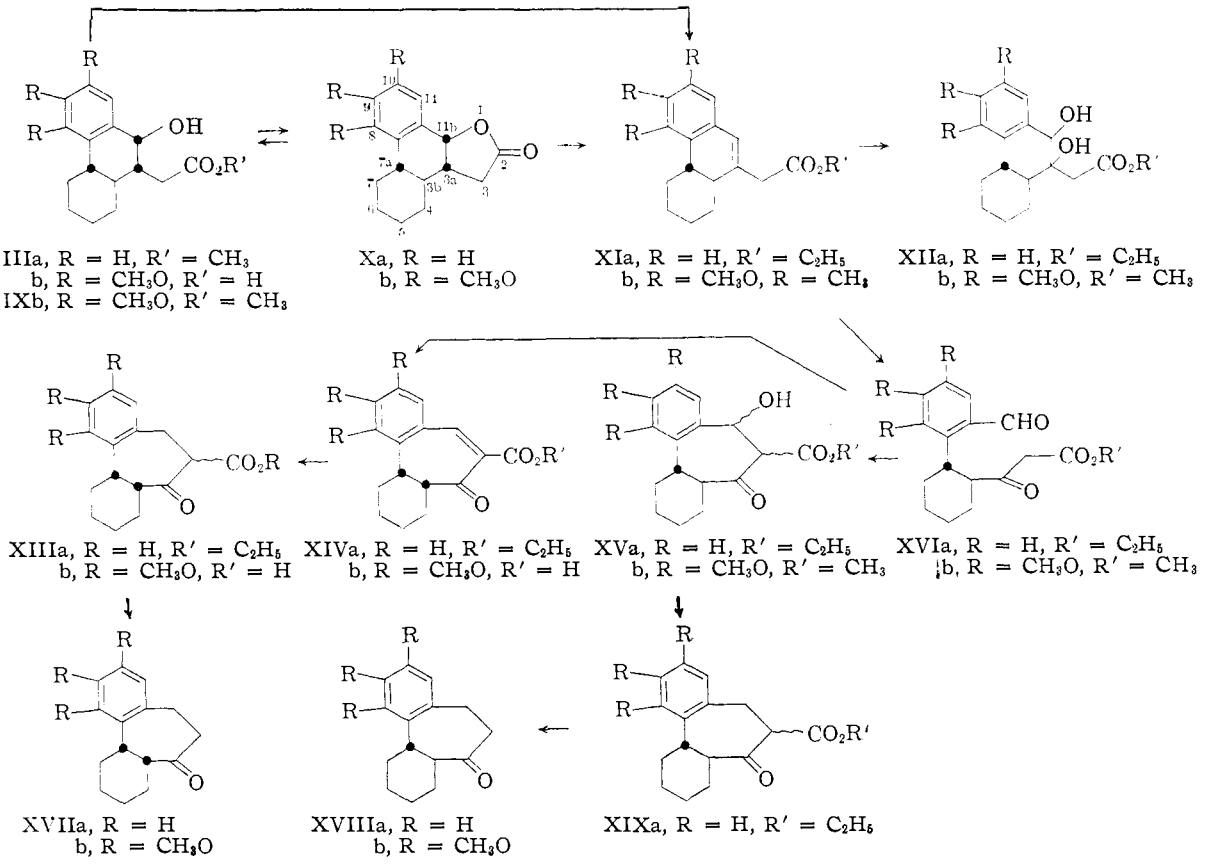
(4) (a) J. Schreiber, W. Leimbruger, M. Pesaro, P. Schudel and A. Eschenmoser, *Angew. Chem.*, **71**, 637 (1959); (b) E. E. van Tamelen, T. A. Spencer, D. S. Allen and R. L. Orvis, *J. Am. Chem. Soc.*, **81**, 6341 (1959).

(5) From one of the reduction mixtures resulting from the action of sodium borohydride on the keto ester IVa it was possible to isolate in 15% yield *via* chromatography on alumina the hydroxy ester VIIIa. This material, for which the sample and the analytical data are unfortunately missing, had m.p. 85–86° and could be dehydrated to XIa in 70% yield by treatment with phosphorus pentoxide in benzene.

(6) *Cf.* A. L. Wilds, "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1944, Vol. II, p. 178 (192) for other examples of the obtention of lactones from Meerwein–Pondorf reactions of γ-keto esters.

(7) (a) J. H. Brewster and C. H. Kucera, *J. Am. Chem. Soc.*, **77**, 4564 (1955); (b) W. G. Dauben, W. K. Hayes, J. S. P. Schwarz and J. W. McFarland, *ibid.*, **82**, 2232 (1960); (c) T. Kanzawa, H. Kamio, M. Sumi and M. Nishikawa, *ibid.*, **80**, 3705 (1958).

(8) That the position of the lactone absorption in the infrared is not an infallible guide to configuration, however, is illustrated by compounds A (*trans*-lactone), B (*cis*-lactone) and C (*trans*-lactone) which

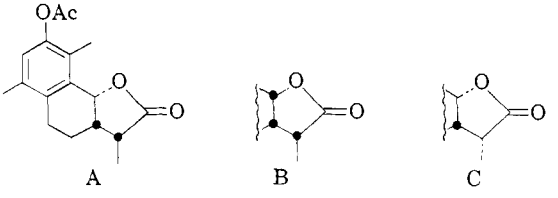


the data of Dauben and Noyce, *et al.*,[9] the reduction of substituted cyclohexanones with sodium borohydride or with aluminum isopropoxide often favors the product of kinetic control ("steric approach control"). On this basis, also, a *cis* configuration for lactone Xa would be predicted.[10]

Employing the method of Cason[11] the lactone Xa was converted to the unsaturated ester XIa in *ca.* 60% yield. The position of the double bond in XIa is indicated by the ultraviolet spectrum which shows an absorption band at 265 mμ (ε 13,000) in close agreement with that reported for dihydronaphthalene[12] (λ_{max} 262 mμ (ε 10,000)).

Attempts to oxidize the olefinic ester XIa by hydroxylation and glycol cleavage to XVIa (or condensation products resulting therefrom) were unsuccessful. The glycol XIIa could not be isolated from osmium tetroxide treatment of XIa, nor could oxidation be effected by the combined hydroxylating–cleavage procedure recently described.[13] The olefinic ester did undergo ozonolysis, however, and the ozonide resulting from the uptake of one mole-equivalent yielded the seven-membered B ring compounds XIVa or XIXa depending upon the method used to decompose it. When an ethyl acetate solution of the ozonide was hydrogenated in the presence of palladium-on-charcoal–palladium hydroxide-on-calcium carbonate catalyst, one mole-equivalent of hydrogen was rapidly absorbed and it is postulated that compound XVIa and/or XVa is formed (in the trimethoxy series, as described in the next section, a compound corresponding to XVa was isolated and characterized). The mixture was then allowed to stand in the presence of a small amount of piperidine for 2 days, and a second hydrogenation was carried out during which another mole-equivalent of hydrogen was absorbed. The resulting oily product is thought to have structure XIXa on the basis of its ultraviolet and infrared spectra which indicate the presence of a non-conjugated benzene ring and a β-keto ester group, respectively. Saponification followed by acidification (decarboxylation) and distillation yielded XVIIIa as an oil from which a solid oxime (as a hydrate) could be obtained in poor yield. The proof of structure of XVIIIa is based on a carbon and hydrogen analysis of the oxime hydrate, on the ultraviolet spectrum (transparent except for benzene absorp-

have absorptions at 1788, 1766 and 1766 cm.$^{-1}$, respectively [W. Cocker and T. B. H. McMurry, *J. Chem. Soc.*, 4430 (1955)].



(9) W. G. Dauben, G. F. Fonken and D. S. Noyce, *J. Am. Chem. Soc.*, **78**, 2579 (1956); W. G. Dauben, E. J. Blanz, J. Jiu and R. A. Micheli, *ibid.*, **78**, 3752 (1956).

(10) A well-documented discussion and review of the chemistry of some related lactone systems has been written by W. Cocker and T. B. H. McMurry, *Tetrahedron*, **8**, 181 (1960).

(11) J. Cason, C. E. Adams, L. L. Bennett and U. D. Register, *J. Am. Chem. Soc.*, **66**, 1764 (1944).

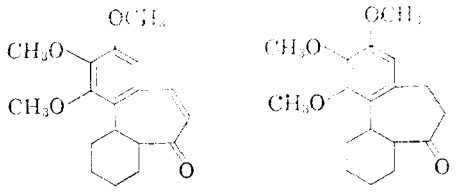
(12) R. A. Morton and A. J. A. DeGauveria, *J. Chem. Soc.*, 916 (1934).

(13) E. von Rudloff, *Can. J. Chem.*, **33**, 1714 (1955).

tion), on the infrared spectrum (absorption at 1706 cm.$^{-1}$ characteristic of a cycloheptanone[14]) and by interrelation with VI, a compound known to have a seven-membered B-ring and known to possess the *trans* configuration between rings B and C.[15] The interrelation was carried out in the following manner: the ester VI was reduced with lithium aluminum hydride to the corresponding carbinol, and this was dehydrated by pyrolysis of the carbinol acetate; ozonolysis of the resulting exocyclic olefin yielded an oily ketone from which a solid oxime hydrate was prepared. This proved to be identical with the oxime hydrate obtained from XIa by the route described above.

Reduction of the ozonide from XIa in a different fashion yielded XVIIa rather than XVIIIa. Thus, when the ozonide was refluxed with water containing suspended zinc powder an oil was obtained from which no pure product could be isolated. Slow distillation of this oil under vacuum, however, yielded 20% of a solid to which structure XIVa is assigned. This formulation is based on a carbon and hydrogen analysis compatible with XIVa, on the presence of an ultraviolet absorption band at 293 mμ (ϵ 12,500) characteristic of a conjugated system of the type present in XIVa,[16] and on the absorption bands at 1712 and 1684 cm.$^{-1}$ in the infrared characteristic of conjugated ester and conjugated ketone carbonyl groups, respectively.[17] The unsaturated keto ester XIVa rapidly absorbed one mole-equivalent of hydrogen in the presence of palladium-on-charcoal catalyst to yield the corresponding saturated keto ester XIIIa as an oil. This was characterized solely by its ultraviolet spectrum which showed absorption only for an isolated benzene ring and by its infrared spectrum which was typical for that of a β-keto ester. Saponification followed by acidification (decarboxylation) yielded XVIIa as an oil from which a solid oxime was obtained. This oxime was different from that obtained from the previous sequence (shown to be *trans*) and is accordingly assigned the *cis* configuration.

The stereochemical outcome of the two methods employed for reducing (and cyclizing) the ozonide can be rationalized on the basis of observations in a closely related series. Loewenthal[18] found that compound XX is more stable in the *cis* configuration, whereas the hydrogenated product XXI is more stable in the *trans* configuration. We postulate, therefore, that in the first sequence described above (XIa → XVa → XIXa → XVIIIa) there is at no time a double bond in the B-ring but that the



XX XXI

intermediate aldol XVa is hydrogenolyzed directly to XIXa; thus, the *trans* configuration of the starting olefin XIa is preserved. In the second sequence described above (XIa → XIVa → XIIIa → XVIIa), on the other hand, the keto ester XIVa possesses a double bond in ring B and, according to Loewenthal's findings, is more stable in the *cis* configuration. The mode of formation of XIVa in this sequence is obscure, but it is felt that this compound is produced during the distillation of the ozonide reduction mixture; it is quite possible that these are conditions sufficient to induce epimerization at the carbon adjacent to the ketone group and, indeed, it is probably this inversion of configuration that allows the formation of the unsaturated keto ester XIVa.

Preparation and Stereochemistry of IVb.—Following procedures similar to those worked out for the unmethoxylated case,[8] 2-(2′,3′,4′-trimethoxyphenyl)-cyclohexanone[19] was condensed with dimethyl succinate to yield a mixture of unsaturated half-esters. These were not separated but were directly hydrogenated to the saturated half-esters Ib, and two isomers (designated as α-Ib and β-Ib) were isolated as crystalline solids. That these isomers differ only in configuration at the carbon α to the carbomethoxy group is shown by the isolation of the same keto acid (Vb) from both α-Ib and β-Ib after cyclization and saponification. That the configuration of these isomers is *trans* on the cyclohexane ring is shown by the following conversion: using previously described methods[15,20] Ib was converted to the reverse half-ester XXII and this to the azide XXIII; thermal rearrangement of the azide followed by hydrolysis[21] yielded the amine XXV which readily lost ammonia to

(14) *Cf.* C. D. Gutsche and T. D. Smith, *J. Am. Chem. Soc.*, **82**, 4067 (1960), for the carbonyl absorptions of common ring ketones obtained on the same instrument under comparable conditions.

(15) C. D. Gutsche, M. W. Wendt and K. L. Seligman, *ibid.*, **80**, 3711 (1958).

(16) Compound XIVa possesses two extended conjugated systems, one a *cis*-benzalacetone type, the other a *trans*-cinnamic acid type. Which is responsible for the band at 293 mμ is conjectural, but on the basis of the following argument the former is suggested. *trans*-Cinnamic acid has λ_{max} 273 mμ (ϵ 21,900) (*cf.* A. E. Gillam and E. S. Stern, "Electronic Absorption Spectroscopy," 2nd ed., Edward Arnold, London, 1957, p. 269, for these and other data pertaining to this discussion) while the 21-benzal derivatives (configuration probably *trans*) of several 20-keto steroids have λ_{max} 294 mμ (ϵ *ca.* 24,000) (W. P. Long, C. W. Marshall, and T. F. Gallagher, *J. Biol. Chem.*, **165**, 197 (1946); C. W. Marshall and T. F. Gallagher, *ibid.*, **179**, 1265 (1949)). It is a quite general phenomenon that while the position of absorption may change in a random fashion when the configuration of an α,β-unsaturated carbonyl system changes from *trans* to *cis*, the intensity of absorption invariably diminishes. Thus, *cis*-cinnamic acid has λ_{max} 264 mμ (ϵ 9500); *trans*-$CH_3CH{=}CHCH{=}CHCHO$ has λ_{max} 270 mμ (ϵ 26,500), while its cyclic *cis* relative 2-formyl-1,1,3-trimethylcyclohexa-2,4-diene has λ_{max} 300 mμ (ϵ 19,900), etc. The position of the absorption band in XIVa is thus in accord with a benzalacetone-type of chromophore and its reduced intensity is in accord with the *cis* configuration.

(17) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1958.

(18) H. J. E. Loewenthal, *J. Chem. Soc.*, 1367 (1958).

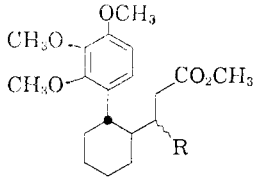
(19) C. D. Gutsche and F. A. Fleming, *J. Am. Chem. Soc.*, **76**, 1771 (1954).

(20) W. S. Johnson and A. Goldman, *ibid.*, **66**, 1030 (1944).

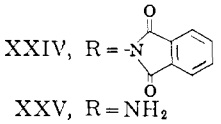
(21) Treatment of the isocyanate with phthalic anhydride (*cf.* K. Brunner, *Ber.*, **47**, 2671 (1914), for a similar reaction and for earlier references including one to an 1854 paper by A. Wurtz) yielded the phthalimido derivative XXIV. One of the many alternative methods investigated for constructing compounds of type VII involved an attempted cyclization of XXIV. Polyphosphoric acid at *ca.* 80° for 5 hr. gave recovered starting material in high yield: polyphosphoric acid at 120° for 30 min. gave a deep red product which could not be purified or identified; saponification of XXIV to the phthalamido acid followed by polyphosphoric acid treatment yielded intractable products.

give the unsaturated acid XXVI; hydrogenation of XXVI then yielded the known *trans*-β-[2-(2′,-3′,4′-trimethoxyphenyl) - cyclohexane] - propionic acid (XXVII).[22]

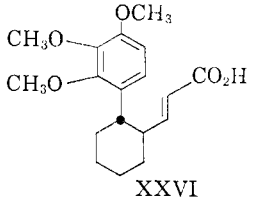
Many procedures for the cyclization of Ib or its corresponding acid chloride were investigated. None of these yielded material of structure II (or III) but all proceeded with ester interchange to yield material of structure IV (or V). The best preparative method for Vb consisted in treating the mixture of α- and β-isomers of Ib with stannic chloride[23] followed by saponification of the resulting mixture of keto esters to a single keto acid; in this fashion Vb could be obtained in 90% over-all yield from Ib. On the basis of conformational considerations similar to those discussed for Va,[15] the keto acid Vb is assumed to have the *trans-trans* configuration.



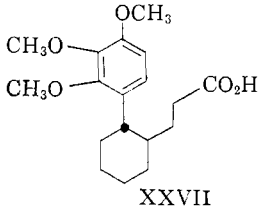
XXII, R = CO_2H
XXIII, R = CON_3



XXIV, R = -N
XXV, R = NH_2



XXVI



XXVII

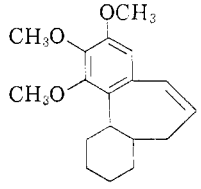
Conversion of Vb to a Seven-membered B Ring Compound.—Reduction of the keto acid Vb with sodium borohydride gave, under careful work up conditions, the hydroxy acid VIIIb which could be quantitatively converted to the hydroxy ester IXb with diazomethane. If, in the processing of VIIIb the reaction mixture is acidified too rapidly, however, the lactone Xb is the only product isolated. Although the lactone cannot (as *was* possible in the unmethoxylated series) be converted to unsaturated ester by the thionyl chloride method (intractable tars formed), it is still usable, for upon re-solution in base followed by careful acidification the hydroxy acid VIIIb reforms. A *cis* configuration is assigned to VIIIb (carbonyl absorption at 1770 cm.$^{-1}$) for reasons similar to those adduced for lactone VIIIa. The conversion of lactone to hydroxy acid almost certainly does not involve a change of configuration at the carbon carrying the hydroxyl group, and it can be inferred that the configuration of VIIIb and IXb is *trans-trans-cis*. Dehydration of the hydroxy ester IXb to the unsaturated ester XIb proceeded smoothly in the presence of phosphorus pentoxide in benzene. The position of the double bond in XIb is indicated by the ultraviolet absorption band at 275 mμ (ε 12,000) characteristic of systems containing a double bond in conjugation with a trimethoxyphenyl ring.[24]

Two general methods for converting XIb to XVIb (or condensation products resulting therefrom) were investigated. Hydroxylation of XIb with osmium tetroxide provided the glycol ester XIIb in good yield. Cleavage of XIIb with periodic acid or lead tetraacetate, however, did not proceed smoothly, and no pure products could be isolated. This can probably be ascribed to the susceptibility of the cleavage product XVIb to further oxidation at the active methylene group.[25] The alternative procedure of converting XIb to XVIb by ozonolysis proved to be the method of choice. Thus, ozonolysis of XIb in ethyl acetate at 0° followed by reductive cleavage with potassium iodide[26] yielded an oil possessing an infrared spectrum commensurate with that expected for XVIb. This material was not purified but was treated with anhydrous hydrogen chloride in benzene[27] and thereby converted to the intramolecular aldol product XVb, obtained as a crystalline solid. Saponification of XVb yielded an unsaturated acid for which the structure XIVb is suggested on the basis of the ultraviolet spectrum which shows λ_{max} 335 mμ (ε 10,800) characteristic of a system containing extended conjugation[28] and the infrared spectrum which shows bands at 1708 and 1695 cm.$^{-1}$ indicating a conjugated carboxyl group and a conjugated keto group, respectively. Further proof for this structure as well as an indi-

(22) When this acid was first prepared it was tentatively assigned the *cis* configuration on the basis of several items of indirect evidence.[19] More recent and more direct evidence indicates that this acid actually has the *trans* configuration.[18]

(23) M. S. Newman, H. V. Anderson and K. H. Takemura, *J. Am. Chem. Soc.*, **75**, 347 (1953).

(24) For instance, the *cis* and *trans* isomers of D have λ_{max} (ε) of 263 mμ (13,100) and 260 mμ (8950), respectively.[18]

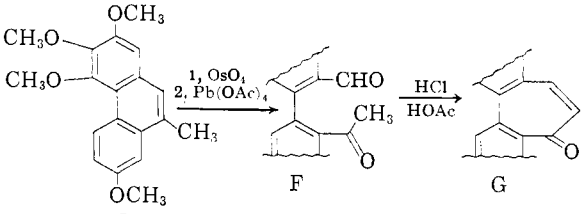


D

(25) C. F. Huebner, S. R. Ames and E. C. Bubl, *J. Am. Chem. Soc.*, **68**, 1621 (1946), have shown that activated methylene groups are susceptible to attack by periodic acid or lead tetraacetate. For instance, ethyl acetoacetate was shown to take up two moles of periodate in one hour and three moles in five hours, the products being carbon dioxide, acetic acid, and formic acid.

(26) P. S. Bailey, *ibid.*, **78**, 3811 (1956).

(27) The conversion of XI *via* XVI to XV (or XIV) is similar in principle to and derives its origin from the conversion of compound E to compound G *via* the intermediate keto aldehyde F as described by J. W. Cook, J. Jack, J. D. Loudon, G. L. Buchanan and J. McMillan, *J. Chem. Soc*, 1397 (1951). It was discovered by these workers that anhydrous hydrogen chloride was the cyclizing agent of choice.



E F G

(28) The unmethoxylated compound XIVa has λ_{max} 293 mμ (ε 12,500). In general, the trimethoxy analogs show a bathochromic shift in their ultraviolet spectra as, for instance, the unmethoxylated keto ester IVa with λ_{max} 249 mμ (ε 12,100)[1] and the methoxylated keto ester IVb with λ_{max} 274 mμ (ε 9800).

cation of its stereochemistry is provided by the conversion to XVIIb.

Not unexpectedly,[29] the unsaturated acid XIVb was completely resistant to decarboxylation even under rather strenuous conditions. After hydrogenation of XIVb to XIIIb, however, decarboxylation took place readily to give XVIIb, identical with a product prepared by Loewenthal[18] by a different route.[30] The obtention of the *cis* isomer is rationalized in terms similar to those suggested for the unmethoxylated series, *viz.*, a base-catalyzed epimerization of the *trans* compound to the *cis* compound to accommodate the generation of a double bond in the seven-membered B ring.

Experimental[31,32]

Unmethoxylated Series

2,3,3a,3b,4,5,6,7,7a,11b-Decahydrophenanthro[9,10-b]-furan-2-one (X). (A) By Aluminum Isopropoxide Reduction.—A 16.7-g. sample of 1,2,3,4,4a,9,10,10a-octahydro-9-ketophenanthryl-10-acetic acid (Va) was refluxed for 2 hr. with 45 g. of aluminum isopropoxide in 850 ml. of xylene. The crude product was recrystallized from ether to give 7.32 g. (46%) of colorless crystals, m.p. 166–167°. An analytical sample was obtained as a colorless micro-crystalline powder after several additional recrystallizations from ether; m.p. 169–170°, $\tilde{\nu}^{CHCl_3}$ 1761 cm.$^{-1}$, $\tilde{\nu}^{KBr}$ 1763 cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49. Found: C, 79.12; H, 7.58.

(B) By Sodium Borohydride Reduction.—A 2.2-g. sample of Va was dissolved in 33 ml. of methanol, the solution was made basic to phenolphthalein with 10% sodium hydroxide, the mixture was cooled in an ice-bath, and 1.8 g. of sodium borohydride in 60 ml. of methanol was added. The resulting product was recrystallized from ether to give 1.45 g. (70%) of Xa, m.p. 166–167°.

Ethyl 1,2,3,4,4a,10a-Hexahydrophenanthryl-10-acetate (XIa).—A 7.32-g. sample of the lactone Xa was refluxed for 3 hr. with 4 ml. of thionyl chloride in 175 ml. of benzene. The mixture was cooled, treated with 230 ml. of absolute ethanol, refluxed for 20 min., and evaporated to leave a crystalline residue. Recrystallization from ether–petroleum ether (b.p. 35–53°) yielded 4.83 g. (59.6%) of tan needles, m.p. 78.5–80.5°. Evaporative distillation followed by recrystallization from ethanol–water gave 4.10 g. (51%) of colorless, very small needles, m.p. 82–82.5°, λ_{max}^{EtOH} 265 mμ (ε 13,000).

Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 80.15; H, 8.06.

***cis*-Ethyl 7a,8,9,10,11,11a-Hexahydro-7-keto-7H-dibenzo[a,c]cycloheptatriene-6-carboxylate (XIVa).**—An 0.800-g. sample of the unsaturated ester XIa dissolved in 40 ml. of chloroform containing 2 ml. of methanol was subjected to ozonolysis at −20° until one mole-equivalent of ozone had been absorbed. The solution was poured into 200 ml. of water, treated with 3 g. of zinc dust, and refluxed for 90 min. The product, obtained by ether extraction, was distilled in a short path apparatus[33] (air bath temperature of 135°, 0.0001 mm. pressure) to give a colorless oil which crystallized from petroleum ether (b.p. 63–69°) as 0.210 g. (21%) of colorless, small needles, m.p. 93–95°. Further recrystallization from the same solvent gave material with m.p. 98–99°; λ_{max}^{EtOH} (ε) 213 mμ (13,600), 237 mμ (12,200), 293 mμ [12,500]; $\tilde{\nu}^{CHCl_3}$ 1712, 1684, 1618 cm.$^{-1}$.

Anal. Calcd. for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09. Found: C, 76.09; H, 7.04.

***cis*-6,7,7a,8,9,10,11,11a-Octahydro-7-keto-5*H*-dibenzo-[a,c]-cycloheptatriene (XVIIa).**—Hydrogenation of XIVa at atmospheric pressure with 10% palladium-on-charcoal catalyst yielded the saturated keto ester XIIIa as a colorless oil: λ_{max}^{EtOH} 263 mμ (ε 2000); $\tilde{\nu}$ 1748 (ester carbonyl), 1709 (ketone carbonyl), 1639 (chelated carbonyl), 1613 cm.$^{-1}$ (double bond).[34] The saturated keto ester was dissolved in a 5% potassium hydroxide solution in 85% aqueous ethanol, and the solution was allowed to stand at room temperature for 2 hr. The solution was then made acidic with sulfuric acid and warmed on the steam-bath for 15 min. (carbon dioxide evolution). After distillation in a short path apparatus[33] (air-bath temperature 105°, 0.0001 mm. pressure) the product consisted of an oil (single sharp carbonyl absorption at 1706 cm.$^{-1}$) which was converted to an oxime obtained as colorless, very small needles after several recrystallizations from aqueous ethanol; m.p. 170–171°. The yield of crystalline oxime from the unsaturated keto ester was *ca.* 25%.

Anal. Calcd. for $C_{15}H_{19}NO$: C, 78.56; H, 8.35. Found: C, 78.33; H, 8.21.

***trans*-6,7,7a,8,9,10,11,11a-Octahydro-7-keto-5*H*-dibenzo[a,c]-cycloheptatriene (XVIIIa). (A) From Ethyl 1,2,3,4,4a,10a-Hexahydrophenanthryl-10-acetate (XIa).**—A 0.400-g. sample of XIa was dissolved in 20 ml. of ethyl acetate containing 1 ml. of absolute methanol, and the solution was cooled to −20° and treated with ozone (2% ozone in oxygen at a rate of 8.4 millimoles of ozone per hr.) until one mole-equivalent had been absorbed. The solution was then added to a suspension of 0.050 g. of 10% palladium-on-charcoal and 0.200 g. of 5% palladium hydroxide-on-calcium carbonate in 20 ml. of ethyl acetate, and the hydrogenation was continued until one mole-equivalent of hydrogen had been absorbed (5 min.). The catalysts were removed by filtration, the filtrate was diluted to 200 ml. with ethyl acetate and treated with 1 ml. of piperidine, and the mixture was allowed to stand at 5° for 2 days. Evaporation of the solvent left an oil which had infrared absorption bands at 3360 (hydroxyl), 1738 (ester carbonyl) and 1709 cm.$^{-1}$ (ketone carbonyl) in accord with a compound of structure XVa. This material was taken up in 30 ml. of ethyl acetate, treated with 0.300 g. of 10% palladium-on-charcoal catalyst and 0.3 ml. of 70% perchloric acid, and hydrogenated at room temperature and atmospheric pressure. After 10 min. the theoretical amount of hydrogen for one mole-equivalent had been absorbed, and the uptake ceased. The crude product was distilled in a short path apparatus[33] (air-bath temperature 140°, 0.1 mm. pressure) to give 0.264 g. of an oil (probably XIXa) which lacked an infrared absorption band at 3360 cm.$^{-1}$ (hydroxyl) but which still had bands at 1742 (ester carbonyl) and 1713 cm.$^{-1}$ (ketone carbonyl). This material was saponified (20 hr. at room temperature with 5% potassium hydroxide in aqueous ethanol) and decarboxylated (1 hr. on steam-bath) as described above for the *cis* isomer, and the resulting product was distilled in a short path apparatus[33] to give 0.072 g. of a colorless, mobile oil showing a single sharp carbonyl absorption at 1706 cm.$^{-1}$. Conversion to the corresponding oxime gave an oil which slowly crystallized from petroleum ether (b.p. 33–58°) to provide a low yield of colorless plates, m.p. 74–76°. The analysis of this material indicates it to be the monohydrate of the oxime of XVIIIa.

Anal. Calcd. for $C_{15}H_{19}NO.H_2O$: C, 72.84; H, 8.56. Found: C, 72.31; H, 8.59.

A semicarbazone of XVIIIa was prepared by the sodium acetate method[35] and, after several recrystallizations from

(29) Facile decarboxylation of β-keto acids requires an intermediate enol species (*cf.*, for example, F. H. Westheimer and W. A. Jones, *J. Am. Chem. Soc.*, **63**, 3283 (1941)), and non-enolizable β-keto acids consequently do not lose carbon dioxide under ordinary conditions (*cf.*, for example, A. C. Cope and M. E. Synerholm, *ibid.*, **72**, 5228 (1950)). The intermediate enol from XIVa would place an allenic system in a seven-membered ring, clearly an exceedingly highly strained structure.

(30) We are indebted to Dr. H. J. E. Loewenthal of the Israel Institute of Technology, Haifa, Israel, for substantiating this identity *via* a mixed melting point determination.

(31) All melting points are corrected; all boiling points are uncorrected.

(32) The ultraviolet spectra were obtained on a Cary recording spectrophotometer; the infrared spectra were obtained on a Perkin–Elmer model 21 recording spectrophotometer.

(33) The short path apparatus consisted of a 2- or 3-bulb distilling unit blown from 8–10 mm. dia. glass tubing, the end bulb containing the sample and being heated in an electrically-controlled air-bath and the second and third bulbs acting as collectors and being cooled by air or by pieces of wet cotton.

(34) N. J. Leonard, H. S. Gutowsky, W. J. Middleton and E. M. Petersen, *J. Am. Chem. Soc.*, **74**, 4070 (1952).

(35) R. L. Shriner, R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 218.

aqueous ethanol, melted at 172.5–173°. Analysis showed that the substance left an inorganic ash on ignition (occlusion of sodium acetate ?), however, and continued recrystallization failed to remove this.

(B) **From *trans-trans*-Methyl 6,7,7a,8,9,10,11,11a-Octahydro-5-keto-5*H*-dibenzo[a,c]cycloheptatriene-7-carboxylate (VIa).**—A 0.500-g. sample of VIa was reduced with lithium aluminum hydride in ether solution to the corresponding carbinol (hydroxyl band in infrared at 3250 cm.$^{-1}$). The carbinol was acetylated with acetic anhydride–pyridine, and the acetate was distilled under reduced pressure (0.001 mm.) through a 0.6 × 25 cm. glass helix-packed tube heated to 575°. The product was a pale yellow oil showing a strong infrared absorption band at 885 cm.$^{-1}$ characteristic of a $R_1R_2C{=}CH_2$ group.[17] Ozonolysis of this material followed by oximation of the resulting product yielded a small amount of the oxime hydrate of XVIIIa, m.p. 73–75°. A mixed m.p. with the oxime hydrate of XVIIIa prepared from XIa as described above in (A) showed no depression in m.p.

Trimethoxy Series

β-Carbomethoxy-β-2-(2′,3′,4′-trimethoxyphenyl)-cyclohexanepropionic Acid.—A slurry of 203 g. (0.77 mole) of 2-(2′,3′,4′-trimethoxyphenyl)-cyclohexanone[19] in 256 g. (1.75 moles) of dimethyl succinate was added to a solution of 50.7 g. (1.3 moles) of potassium in 900 ml. of absolutely dry *t*-butyl alcohol.[36] The system was filled with nitrogen, and the mixture was refluxed for 3 hr. The solution was then cooled in ice, acidified, and worked up in the usual way to give 232 g. (80%) of a light amber oil; λ_{max}^{EtOH} (ε) 238 mμ (3810), 272 mμ (1060). Further purification was not attempted, but the crude product was used directly in the following experiment.

α-Isomer of β-Carbomethoxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionic Acid (α-Ib).—A solution of 62.1 g. of crude Stobbe product (*cf.* above) in 400 ml. of absolute methanol was treated with 14 g. of 10% palladium-on-charcoal and hydrogenated for 48 hr. at 2 atmospheres of pressure. The product consisted of 60 g. (96%) of a mixture of epimers from which a pure epimer (designated as the α-isomer) was isolated in 13% yield after many recrystallizations from petroleum ether (b.p. 80–110°)–ethyl acetate (ratio of 9:1) followed by ethyl acetate alone; m.p. 176–177°; λ_{max}^{EtOH} (ε) 225 mμ (10,500), 276 mμ (1050).

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.21; H, 7.16.

β-Isomer of β-Carbomethoxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionic Acid (β-Ib).—Concentration of the mother liquors from the recrystallization which yielded the α-isomer gave a solid which was recrystallized several times from aqueous ethanol to produce 13% of another epimer (designated as the β-epimer), m.p. 110–111°; λ_{max}^{EtOH} (ε) 221 mμ (10,300), 275 mμ (980).

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 62.64; H, 7.34.

α-Isomer of β-Carboxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionic Acid.—Saponification of α-Ib with 5% aqueous sodium hydroxide (2 hr. at reflux) yielded 100% of material, m.p. 182–186°, which was purified by several recrystallizations from aqueous ethanol; m.p. 188.5–190°. Analysis indicated it to be a monohydrate.

Anal. Calcd. for $C_{19}H_{26}O_7 \cdot H_2O$: C, 59.36; H, 7.34. Found: C, 59.37; H, 7.22.

A sample which had been heated at 130° under vacuum for several hours was analyzed.

Anal. Calcd. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.26; H, 7.14.

β-Isomer of β-Carboxy-β-[2-(2′,3′,4′-trimethoxyphenyl) cyclohexane]-propionic Acid.—Saponification of β-Ib with 5% aqueous sodium hydroxide (2 hr. at reflux) yielded 79% of material, m.p. 171–173°, which was purified by several recrystallizations from aqueous ethanol; m.p. 171–172°.

Anal. Calcd. for $C_{19}H_{26}O_7$: C, 62.28; H, 7.15. Found: C, 62.25; H, 7.07.

(36) It is important to use very dry alcohol in this condensation. In the present instance ordinary *t*-butyl alcohol was purified by distilling twice from sodium wire and once from potassium followed by fractionation through a 12-in. Vigreux column, the fraction boiling at 81–83° being collected.

α-Isomer of Methyl β-Carboxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionate (α-XXII).—An 8.8-g. sample of the α-isomer of β-carboxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionic acid (*cf.* above) was refluxed for 2 hr. with 25 ml. of acetyl chloride. The excess acetyl chloride was removed under vacuum, and the residue was refluxed another 2 hr. with 25 ml. of absolute methanol. The product was obtained as 5.3 g. (60%) of solid, m.p. 120–125°, which yielded colorless, very fine needles, m.p. 132–133°.

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.39; H, 7.85.

β-Isomer of Methyl β-Carboxy-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionate (β-XXII).—Using the same procedure as described above for the α-isomer, the β-isomer of β-carboxy-β-[2-(2′,3′4′-trimethoxyphenyl)-cyclohexane]-propionic acid was converted to the corresponding reverse half-ester and obtained, after recrystallization from petroleum ether (b.p. 63–69°), as colorless, very small crystals, m.p. 122–123°.

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found C, 62.93; H, 7.64.

Methyl β-Carboxy-β-[2-(2′,3′,4′-trimethoxy-5′-bromophenyl)-cyclohexane]-propionate.—A suspension of 1.0 g. (2.08 mmoles) of the silver salt from α-XXII in 5 ml. of chloroform was treated with 0.66 g. (4.14 mmoles) of bromine in 8 ml. of chloroform. The neutral product consisted of a solid, m.p. 161–163°, which was recrystallized from benzene–hexane to give colorless flakes, m.p. 163–164°.

Anal. Calcd. for $C_{20}H_{27}BrO_7$: C, 52.30; H, 5.92. Found: C, 52.48; H, 6.27.

β-Carboxy-β-[2-(2′,3′,4′-trimethoxy-5′-bromophenyl)-cyclohexane]-propionate.—Saponification of the bromine-containing half-ester described above yielded the corresponding dibasic acid as a colorless, microcrystalline powder after recrystallization from benzene–hexane; m.p. 194–195°.

Anal. Calcd. for $C_{19}H_{25}BrO_7$: C, 51.24; H, 5.66. Found: C, 51.10; H, 5.80.

Methyl β-Phthalimido-β-[2-(2′,3′,4′-trimethoxyphenyl)-cyclohexane]-propionate XXIV).—A 3.0-g. sample of α-XXII was converted to the corresponding acid chloride by treatment of the sodium salt with oxalyl chloride[37] and then to the azide α-XXIII with sodium azide in aqueous acetone.[38] The azide was refluxed for 30 min. in 25 ml. of benzene (nitrogen evolution), the solvent was removed, and the residue was mixed with 10 g. of phthalic anhydride and 3 drops of quinoline and heated for 2 hr. at 180–200°. Chromatography on alumina of the resulting product yielded 1.2 g. (32%) of the phthalimido compound XXIV which, after several recrystallizations from ether–hexane, was obtained as colorless, very small rectangular plates, m.p. 103–104°.

Anal. Calcd. for $C_{27}H_{31}NO_7$: C, 67.34; H, 6.49; N, 2.91; CH_3O, 25.77. Found: C, 67.20; H, 6.51; N, 3.10; CH_3O, 25.22.

***trans*-β-2-(2′,3′,4′-Trimethoxyphenyl)-cyclohexanepropionic Acid (XXVII).**—A 1.0-g. sample of α-XXII was carried through the reactions described above to the removal of solvent after the refluxing in benzene. The residue was dissolved in 15 ml. of dioxane and 4 ml. of water, and the solution was refluxed for 12 hr. The product from this treatment, an amorphous powder with m.p. 72–91° thought to be the amino acid XXV, was refluxed for 3 hr. with an aqueous alcoholic solution of potassium hydroxide. The resulting material (probably XXVI) was hydrogenated over 10% palladium-on-charcoal catalyst, and from the hydrogenation mixture a solid with m.p. 77–79° was isolated. A mixed m.p. with an authentic sample of *trans*-XXVII, m.p. 81–83°, was 79–82°.

Methyl 1,2,3,4,4a,9,10,10a-Octahydro-5,6,7-trimethoxy-9-keto-phenanthrylacetate (IVb).—A 0.300-g. sample of α-Ib was converted to the acid chloride and cyclized with anhydrous aluminum chloride in carbon disulfide by the procedure previously described for the unmethoxylated analog.[8] The product, obtained in 70% yield, was re-

(37) R. Adams and L. H. Ulich, *J. Am. Chem. Soc.*, **42**, 599 (1920); A. L. Wilds and C. H. Shunk, *ibid.*, **70**, 2427 (1948).

(38) P. A. S. Smith, "Organic Reactions," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 337 (387).

crystallized from aqueous ethanol and obtained as very small, elongated blades, m.p. 87.5–88.5°; λ_{max}^{EtOH} (ϵ) 223 mμ (24,300), 274 mμ (9800), 308 mμ (3930).

Anal. Calcd. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23. Found: C, 66.10; H, 7.15.

1,2,3,4,4a,9,10,10a-Octahydro-5,6,7-trimethoxy-9-ketophenanthryl-10-acetic Acid (Vb). (A) By Saponification of IVb.—Saponification of the pure sample of IVb (see above) yielded a quantitative amount of the corresponding acid which, after recrystallization from ethyl acetate–hexane, melted at 172–174°.

Anal. Calcd. for $C_{19}H_{24}O_6$: C, 65.60; H, 6.94. Found: C, 65.27; H, 6.74.

(B) Directly from Ib without Isolation of IVb.—The best preparative method for Vb employs crude hydrogenated Stobbe half-ester Ib and makes use of the epimerization that occurs during the saponification of the crude keto ester. A mixture of 43 g. (0.113 mole) of Ib (crude product), 25.8 g. (0.124 mole) of phosphorus pentachloride and 200 ml. of dry benzene was warmed on the steam-bath until no more solid remained. The solution was then cooled to 5° and treated, with stirring, with a cold solution of 70.7 g. (0.272 mole) of anhydrous stannic chloride in 100 ml. of benzene. After stirring at 5–10° for 8 min. the mixture was poured onto ice and hydrochloric acid, and the product was isolated in the usual fashion as a semi-solid. This was treated with 200 ml. of an ethanol–water (70:30) solution containing 20 g. of dissolved potassium hydroxide, and the mixture was refluxed for 2–3 hr. The product consisted of 39 g. (99%) of a white solid, m.p. 166–170°, which after two recrystallizations from ethyl acetate–hexane gave material with m.p. 172–174° identical with that described above.

1,2,3,4,4a,9,10,10a-Octahydro-5,6,7-trimethoxy-9-hydroxyphenanthryl-10-acetic Acid (VIIIb).—A 58-g. (0.167 mole) sample of crude keto acid Vb was dissolved in 600 ml. of methanol, the solution was made basic to phenolphthalein with 10% sodium hydroxide solution, and 16 g. of sodium borohydride in 250 ml. of methanol containing a few drops of 10% sodium hydroxide solution was added. After the reaction mixture had stood at room temperature for 16 hr. the methanol was removed under vacuum, and the residue was dissolved in water. To the stirred and cooled (ice) aqueous solution 1 *N* phosphoric acid was very slowly added from a burett, the tip of which was below the surface of the liquid. When the solution was acidic to brom phenol blue the addition was stopped, and the precipitated acid was removed by filtration and obtained as 55.6 g. (95.5%) of solid, m.p. 188–194°. Three recrystallizations from ethyl acetate–hexane yielded an amorphous white powder, m.p. 201–202°.

Anal. Calcd. for $C_{19}H_{26}O_6$: C, 65.12; H, 7.48. Found: C, 64.99; H, 7.49.

2,3,3a,3b,4,5,6,7,7a,11b-Decahydro-8,9,10-trimethoxyphenanthro[9,10-b]furan-2-one (Xb).—If, in the procedure described above, phosphoric acid is added too rapidly or if 1 *N* sulfuric acid is used in place of phosphoric acid, the lactone Xb is formed in 80–90% yield. It can be obtained as colorless, silky needles after recrystallization from ethyl acetate–hexane; m.p. 181–182°, $\bar{\nu}^{KBr}$ 1770 cm.$^{-1}$ (lactone carbonyl).

Anal. Calcd. for $C_{19}H_{24}O_6$: C, 68.65; H, 7.28. Found: C, 68.76; H, 7.62.

Methyl 1,2,3,4,4a,9,10,10a-Octahydro-5,6,7-trimethoxy-9-hydroxyphenanthryl-10-acetate (IXb).[39]—A 29-g. sample of VIIIb was treated with an excess of ethereal diazomethane to yield 29.4 g. (98%) of the hydroxy ester IXb. Recrystallization from ethyl acetate–hexane gave a 70% yield of colorless, microscopic flakes, m.p. 77.5–78.5°.

Anal. Calcd. for $C_{20}H_{28}O_6$: C, 65.91; H, 7.74. Found: C, 65.77; H, 7.44.

Methyl 1,2,3,4,4a,10a-Hexahydro-5,6,7-trimethoxyphenanthryl-10-acetate (XIb).—A 14.8-g. (0.04 mole) sample of hydroxy ester IXb was dissolved in 125 ml. of dry benzene and treated with 40 g. of phosphorus pentoxide. After stirring at room temperature for 45 min. the reaction mixture was poured onto ice and worked up in the usual fashion to yield a semi-solid. Recrystallization from benzene–hexane gave 9.4 g. (67%) of opaque crystalline cubes, m.p. 76–77°; λ_{max}^{EtOH} (ϵ) 275 mμ (12,100), 307 mμ (3200).

Anal. Calcd. for $C_{20}H_{26}O_5$: C, 69.34; H, 7.57. Found: C, 69.30; H, 7.67.

Methyl 1,2,3,4,4a,9,10,10a-Octahydro-5,6,7-trimethoxy-9,10-dihydroxyphenanthryl-10-acetate (XIIb).—To a solution of 0.495 g. (1.95 mmoles) of osmium tetroxide[40] in 25 ml. of dry ether cooled to 0° was added 0.670 g. (1.95 mmoles) of unsaturated ester XIb in 25 ml. of ether. After standing in the dark for 24 hr. the ether was removed by evaporation, and the residue was taken up in methylene chloride. The solution was shaken for 3 hr. with a 20% solution of sodium sulfite[41] to reduce the osmium complex, and the solvent was evaporated to give an oily residue. Crystallization from ether–hexane gave 0.385 g. (71%) of colorless, opaque, very small needles, m.p. 155–156°.

Anal. Calcd. for $C_{20}H_{28}O_7$: C, 63.14; H, 7.42. Found: C, 63.19; H, 7.44.

Methyl 1,2,3,4,4a,6,7,11b-Octahydro-9,10,11-trimethoxy-5-keto-7-hydroxydibenzo[a,c]cycloheptatriene-6-carboxylate (XVb).—A 0.50-g. sample of the unsaturated ester XIb dissolved in 25 ml. of ethyl acetate was cooled to 0° and treated with oxygen containing *ca.* 2% ozone. The uptake of ozone ceased after one mole-equivalent had passed through the sample, whereupon the reaction mixture was treated with a slight excess of a 10% solution of potassium iodide containing one drop of concentrated sulfuric acid and was mechanically shaken for 0.5 hr. Following this, the mixture was heated with swirling on the steam-bath for 5 min. and then worked up in the usual fashion to give a crude product as an oil; $\bar{\nu}$ 7140 (ester carbonyl), 1710 (ketone carbonyl), 1680 cm.$^{-1}$ (ArCHO carbonyl).[42] This was dissolved in benzene, and the solution was saturated with dry hydrogen chloride and allowed to stand overnight at room temperature. The hydrogen chloride was then removed under vacuum at room temperature followed by extraction of the benzene solution with aqueous sodium bicarbonate solution. Evaporation of the benzene gave 0.44 g. (80%) of XVb, a sample of which was purified for analysis by several recrystallizations from petroleum ether (b.p. 63–69°)–ether and obtained as a colorless, microcrystalline powder, m.p. 218–219°; $\bar{\nu}^{KBr}$ 3300 (hydroxyl), 1736 (ester carbonyl), 1711 cm.$^{-1}$ (ketone carbonyl).

Anal. Calcd. for $C_{20}H_{26}O_7$: C, 63.48; H, 6.93. Found: C, 63.48; H, 6.95.

***cis*-1,2,3,4,4a,11b-Hexahydro-9,10,11-trimethoxy-5-keto-6-carboxydibenzo[a,c]cycloheptatriene (XIVb).**—A 0.50-g. sample of the crude hydroxy-keto-ester XVb from the preceding experiment was saponified by refluxing for 2 hr. with 10 ml. of an ethanol–water mixture (70:30) containing 0.5 g. of potassium hydroxide. The product consisted of 0.22 g. (48%) of solid which, after recrystallization from ether–hexane, was obtained as bright yellow rhombs, m.p. 152–153°; λ_{max}^{EtOH} (ϵ) 237mμ (10, 300), 252 mμ (12,300), 335 mμ (10,800); $\bar{\nu}^{KBr}$ 1708 (C=C—CO_2H carbonyl), 1695 cm.$^{-1}$ (C=C—C=O carbonyl).-

Anal. Calcd. for $C_{19}H_{22}O_5$: C, 65.88; H, 6.40. Found: C, 66.04; H, 6.35.

***cis*-1,2,3,4,4a,6,7,11b-Octahydro-9,10,11-trimethoxy-5-keto-dibenzo[a,c]cycloheptatriene (XVIIb).**—A 0.375-g. sample of the unsaturated acid XIVb was dissolved in 25 ml. of ethanol, treated with 10% palladium-on-charcoal catalyst, and hydrogenated at room temperature until one mole-equivalent of hydrogen had been absorbed (0.5-1 hr.). The catalyst was removed by filtration, the solvent was removed by evaporation, and the residue was dissolved in 10 ml. of a solution of ethanol–water (3:1) which was 0.02 *M* in aniline hydrochloride and 0.012 *M* in hydrochloric acid. The solution was allowed to stand at room tempera-

(39) We are indebted to Mr. Thomas Layloff for his aid in the preparation of substantial amounts of this material.

(40) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *J. Am. Chem. Soc.*, **74**, 4223 (1952).

(41) L. H. Sarett, *ibid.*, **68**, 2478 (1946).

(42) An equimolar mixture of ethyl acetoacetate and 2,3,4-trimethoxybenzaldehyde showed an almost identical spectrum in this region with bands at 1740, 1720 and 1685 cm.$^{-1}$.

ture for 2 hr. and was then heated on the steam-bath for 15 min. The product consisted of 0.265 g. (80%) of a semi-solid which was recrystallized from hexane to yield a product, m.p. 113–115°. Further recrystallization gave 0.165 g. (50%) of XVIIb as colorless needles, m.p. 115–115.5° (lit[18] 115.5°). A mixed m.p. with a sample of XVIIb prepared by a different route[18] showed no depression in m.p.[30]

Anal. Calcd. for $C_{18}H_{24}O_4$: C, 71.02; H, 7.95. Found: C, 71.03; H, 8.16.

[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON 15, MASS., AND THE DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH, ARGONNE NATIONAL LABORATORY, LEMONT, ILLINOIS][1]

Optical Rotatory Dispersion of Dyes Bound to Macromolecules. Cationic Dyes: Polyglutamic Acid Complexes[2,3]

BY LUBERT STRYER AND E. R. BLOUT

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The optical rotatory dispersions of complexes of cationic dyes with synthetic high molecular weight poly-α-glutamic acids have been measured. The dyes used were Acridine Orange, pseudoisocyanine and a substituted toluidine-thiazole dye, [*p*-(5-acetyl-4-methyl-2-thiazolylazo)-N-β-aminoethyl-N-ethyl-*m*-toluidine]. Each of these dyes forms a complex with polyglutamic acid over the *p*H range 4.2 to 6.5. At *p*H's above 5.5 polyglutamic acid is in the random conformation and the dye:polypeptide complexes show optical rotatory dispersion due to the polypeptide alone. At *p*H's below 5.1 polyglutamic acid is in the α-helical conformation and the dye:polypeptide complexes show anomalous rotatory dispersion in the dye absorption band (the Cotton effect). The magnitude of the observed Cotton effects is very high; in some cases the molar rotation is in the range 100,000 to 1,000,000. The inflection points of the induced Cotton effects are in good agreement with the absorption maxima of the dyes which leads to the conclusion that the dye chromophores acquire optical rotatory power upon binding to the helical polypeptides. For a single absorption band the signs of the induced Cotton effect are opposite for poly-α,L-glutamic acid and poly-α,D-glutamic acid. It is therefore concluded that helices of opposite screw-sense (L and D) show Cotton effects of opposite sign. Two models are suggested to explain the observed Cotton effects in dye:helical polypeptide complexes. Possible uses of this method in the elucidation of the structure of macromolecules of unknown conformation are mentioned.

Introduction

Optical rotation has been used in recent years to study the conformations of numerous macromolecules of biological interest.[4] Since helical structures are dissymmetric and contribute to the optical rotation of a macromolecule,[5] they may be characterized by this method. For example, the thermodynamic stability[6] and proportion of helix[7] present in some proteins has been estimated. In addition, the activation[8] and denaturation[9a] of enzymes have been studied by this method. These are significant contributions to our understanding of the macromolecular conformations of synthetic polypeptides and proteins.

Another approach to the determination of the conformations of macromolecules is the study of the optical rotation within the intrinsic absorption bands of the macromolecules. However, most work to date has been limited for technical reasons to wave lengths outside these ultraviolet absorption bands.[9b] In these spectral regions where absorption is low the sum of several optical rotatory contributions has been measured.

A more feasible approach, presented in this paper, involves the measurement of the optical rotatory dispersion throughout the absorption bands of extrinsic chromophores (dyes) bound to synthetic polypeptides. We have reported previously that some symmetric (non-optically active) dye molecules bound to the helical conformation of a polypeptide show an induced anomalous optical rotatory dispersion (Cotton effect) in the absorption band of the bound dye, while no such effect was observed in complexes of dye and the random conformation of the polypeptide.[10]

This paper deals with the optical rotatory dispersion of complexes of three different types of cationic dyes with polyglutamic acid. Induced Cotton effects in the absorption bands of the bound dye have been studied with respect to the helix content of the polypeptide, the relative screw-sense of the helix and some of the conditions which alter the mode of binding. These findings with a model system of known conformation may provide a basis for using this method in studying macromolecules of unknown structure.

Experimental

Polypeptides.—Two synthetic polypeptides were used in this study. The sodium salts of poly-α,L-glutamic acid (L-PGA), sample R4273-112, and of poly-α,D-glutamic acid (D-PGA), sample GF-5-219, were prepared in a manner

(1) A portion of this work was performed under the auspices of the U. S. Atomic Energy Commission, while L. S. was in residence at the Argonne National Laboratory.

(2) This paper is Polypeptides XXXIII. For the preceding paper in this series see T. Miyazawa and E. R. Blout, THIS JOURNAL, **83**, 712 (1961). Alternate address of E. R. Blout, Research Division, Polaroid Corporation, Cambridge 39, Mass.

(3) This work was supported in part by U. S. Public Health Service Grant #A2558.

(4) See for example (a) E. R. Blout, "Optical Rotatory Dispersion," by C. Djerassi, McGraw-Hill Book Company, New York, N. Y., 1960, p. 238; (b) J. R. Fresco, *Trans. N. Y. Acad. Sci.*, **21**, 653 (1959).

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(10) (a) E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci. U. S.*, **45**, 1591 (1959); (b) L. Stryer and E. R. Blout, *Abstracts of the Biophysical Society, 4th Annual Meeting*, February, 1960.