form–nitromethane, or ether gave the same result. Mass spectral data in *m/e* (rel intensity) follow.

Compound **2**: ion chamber 143°; M^+ 436 (9), 376 (100), 361 (13), 251 (41), 235 (12), 209 (16), 197 (9), 181 (10), 158 (2), 155 (10), 143 (6), 128 (4); metastable ion 347.

Compound **3**: ion chamber 134°; 436 (7), 378 (18), 376 (100), 361 (14), 253 (12), 251 (67), 237 (9), 235 (19), 209 (12), 197 (22), 181 (12), 155 (21), 143 (13), 128 (13).

Compound **4**: ion chamber 200°; 436 (2), 434 (14), 376 (9), 374 (100), 359 (10), 249 (64), 235 (25), 233 (19), 209 (10), 207 (11), 195 (7), 179 (11).

Compound **5**: ion chamber 185°; M^+ 562 (0.3), 502 (0.6), 434 (9), 374 (100), 359 (15), 249 (60), 233 (28), 207 (18), 179 (18), 153 (4); metastable ions 165.7, 345.

Compound **8** (containing some **5**): ion chamber 200°; 504 (16), 434 (5), 376 (100), 374 (23), 36 (8), 251 (19), 249 (21), 235 (7), 233 (5), 209 (8), 207 (5), 197 (5), 195 (7), 181 (6), 179 (6), 155 (6), 153 (6); metastable ions 324, 345.

Compound **15**: ion chamber 135°; M^+ 436 (38), 376 (26), 361 (17), 311 (71), 295 (24), 257 (14), 251 (100), 235 (19), 209 (24), 197 (33), 181 (21), 155 (24), 179 (17), 119 (8), 55 (76); metastable ions 202.6, 222.

Compound **16**: ion chamber 190°; M^+ 564 (3), 504 (5.5), 436 (31), 376 (33), 361 (17), 311 (45), 295 (23), 258 (12), 251 (100), 235 (21), 209 (27), 197 (31), 181 (17), 179 (14), 155 (19), 119 (5), 55 (70); metastable ions 202.6, 222.

Compound **20**: ion chamber 180°; M^+ 500 (42), 485 (19), 375 (39), 374 (100), 363 (4), 359 (4), 253 (14), 237 (6), 211 (3), 199 (6), 183 (5), 157 (9), 55 (60); metastable ions 170.5, 222, 280, 322, 345, 471.

Compound **21**: ion chamber 220°; M^+ 628 (2), 613 (0.2), 500 (13), 485 (12), 375 (21), 374 (58), 253 (6), 237 (4), 183 (3), 157 (6), 55 (100); metastable ions 280, 471.

Registry No.—**2**, 1060-56-6; **3**, 21549-35-9; **4**, 21549-36-0; **5**, 26885-77-8; **8**, 36959-76-9; **9**, 36959-77-0; **15**, 36959-78-1; **16**, 36959-79-2; **20**, 36959-80-5; **21**, 36959-81-6; tetracyanoethylene, 670-54-2.

Acknowledgments.—We thank Mr. Jack Eyman for valuable assistance with mass spectra, and the National Science Foundation for providing funds for the mass spectrometer at Kent State University.

Stereochemistry of Some Δ^1-Butenolide Syntheses

GARY S. CHAPPELL

School of Pharmacy, University of Missouri—Kansas City, Kansas City, Missouri 64110

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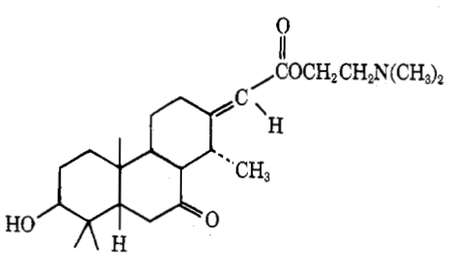
Alkylation of *trans*-1- and *trans*-2-decalone with ethyl bromoacetate *via* the pyrrolidine enamine and subsequent ester hydrolysis and dehydration (Ac_2O) gave equatorially fused butenolides. The Reformatsky products from 3(*a*)-acetoxy-*trans*-2-decalone, ethyl bromoacetate, zinc, and trimethyl borate were saponified and ring closed to give isomeric β-hydroxy γ-lactones which could be dehydrated to the axially fused butenolide. Similar reactions with 3(*e*)-acetoxy-*trans*-2-decalone gave the other two isomeric β-hydroxy-γ-lactones which on dehydration gave the equatorially fused butenolide. Stereochemical assignments were made on the basis of nmr spectra.

Interest in the preparation of conformationally rigid butenolide analogs of cassaine (**1**) such as **2** made



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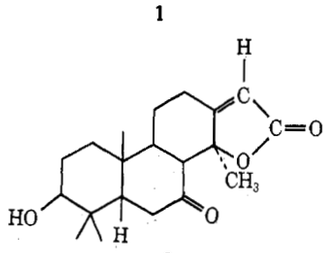
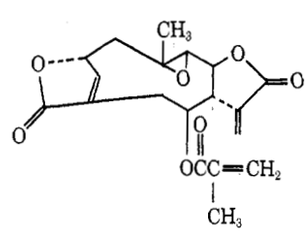


2

necessary the investigation of the stereochemistry of some Δ^1-butenolide syntheses. Of the large number of syntheses for the Δ^1-butenolides most have been applied only to nonfused ring systems.[2] However, in recent years several butenolide syntheses have been used for fused ring systems involved in naturally occurring compounds.[3-10] Further interest in the chemistry of butenolides has been generated by the discovery of naturally occurring fused butenolides with tumor-inhibitor activity such as elephantopin (**3**).[11]



3

The butenolide syntheses investigated were selected because they appeared to have applicability in the synthesis of the desired cassaine analogs. *trans*-Decalin was chosen as the model because it is conformationally rigid and the B–C rings of cassaine are a trans-fused decalin ring system. Thus, synthetic approaches to 2-

(1) This research was supported by Faculty Research Grants and by Assistant Professor Research Grants from the University of Missouri—Kansas City, Mo.

(2) Y. S. Rao, *Chem. Rev.*, **64**, 353 (1964); P. E. Sonnet, *Chem. Ind. (London)*, 1296 (1967).

(3) W. W. Epstein and A. C. Sonntag, *J. Org. Chem.*, **32**, 3390 (1967).

(4) J. N. Marx and F. Sondheimer, *Tetrahedron, Suppl.*, **8**, 1 (1966).

(5) E. Demole and P. Engist, *Helv. Chim. Acta*, **51**, 481 (1968).

(6) H. Minato and T. Nagaski, *J. Chem. Soc.*, 377 (1966).

(7) W. C. Bailey, Jr., A. K. Bose, R. M. Ikeda, R. H. Newman, H. V. Secor, and C. Varsel, *J. Org. Chem.*, **33**, 2819 (1968).

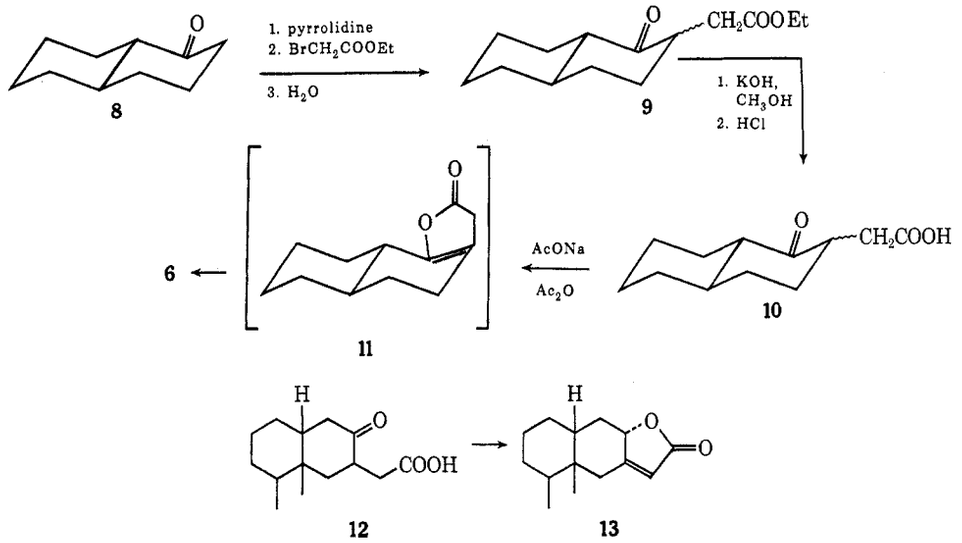
(8) S. W. Pelletier, A. L. Chappell, and S. Probhakar, *J. Amer. Chem. Soc.*, **90**, 2889 (1968).

(9) E. Piers, M. B. Geraghty, and R. D. Smillie, *Chem. Commun.*, 614 (1971).

(10) Z. Horii, M. Ito, I. Minami, M. Yamauchi, M. Hanaoka, and T. Momose, *Chem. Pharm. Bull.*, **18**, 1967 (1970).

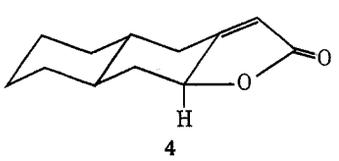
(11) Y. Aynchchi, J. M. Cassady, A. T. McPhail, G. A. Sims, H. K. Schnoes, S. M. Kupchan, and A. L. Burlingame, *J. Amer. Chem. Soc.*, **88**, 3674 (1966).

SCHEME I



8 9 10 11 12 13

[3(*e*)-hydroxy-2-decalylidine]acetic acid γ-lactone (**4**), 2-[3(*a*)-hydroxy-2-decalylidine]acetic acid γ-lactone (**5**), 2-[1(*e*)-hydroxy-2-decalylidine]acetic acid γ-lactone (**6**), and 2-[1(*a*)-hydroxy-2-decalylidine]acetic acid γ-lactone (**7**) were explored. Butenolides **4** and **6** are fused in an equatorial manner, while **5** and **7** are axially fused since the lactone oxygen is equatorial in **4** and **6** and axial in **5** and **7**.



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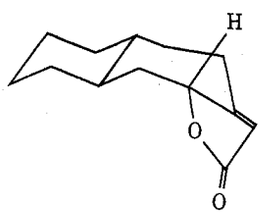

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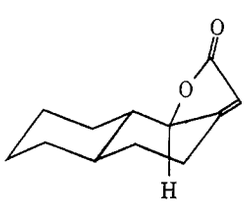

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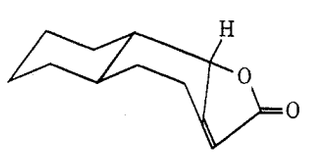


7

The first synthetic approach is outlined in Scheme I and is essentially that of Minato and Nagaski.[6] The equatorially fused butenolides **4** and **6** were obtained utilizing *trans*-2-decalone and *trans*-1-decalone (**8**), respectively.

The equatorial nature was assigned on the basis of nmr spectra. The C-3 proton of **4** appeared at δ 4.65 as a four-line multiplet with further fine splitting. The larger coupling constants were observed as 9 Hz for the axial–axial coupling and 6 Hz for the axial–equatorial coupling with the C-4 protons.[12] The fine splitting was shown by spin–spin decoupling to be due to coupling with the vinyl proton of the butenolide ring. The C-1 proton of **6** was observed at δ 4.39 as a doublet (J = 8 Hz) with further fine splitting. The doublet nature is due to coupling with the axial proton at C-9. The fine splitting is due to coupling with the vinyl proton. No evidence of the axially fused isomers **5** or **7** was observed in their respective reaction sequences. This would be expected, since the lactonization must involve the enol (example **11**), which on isomerization would give the more stable equatorial arrangement. The equatorially fused butenolide was obtained by Piers, *et al.*,[9] in their synthesis of ermophilenolide, which involved lactonization of **12** to **13**. The synthetic approach outlined in Scheme I is an excellent route to the equatorial isomers, since a ketone with no other functionality is the starting material and the yields are reasonable.

The second synthetic approach studied is outlined in Scheme II. The Reformatsky reaction of 3(*e*)-acetoxy-*trans*-2-decalone (**14**) with ethyl bromoacetate was conducted according to the procedure of Rathke and Lindert,[13] which employs trimethyl borate as a Lewis acid to reduce the basicity of the reaction. This procedure gave improved yields over conventional Reformatsky conditions. Column chromatography of the reaction mixture gave approximately a 2:1 ratio of the equatorial addition product **15** to axial addition product **16**. A very small amount of the diol **17** was also obtained, which on acetylation with acetic anhydride gave **15**. The stereochemistry at C-2 of **16** and **17** was assigned by comparison of chemical shifts of hydroxyl protons in deuterated dimethyl sulfoxide.[14] The hydroxyl proton of **16** is 9.4 Hz downfield from the hydroxyl proton of **15**, which is consistent with an equatorial hydroxyl in **16** and an axial hydroxyl in **15**. The magnitude of the difference is considerably less than reported.[14] Further support for this assignment will be presented below. The protons at C-3 had peak widths at one-half height of 18 and 17 Hz for **15** and **16**, respectively, which is consistent for axial protons and thus an equatorial acetate.

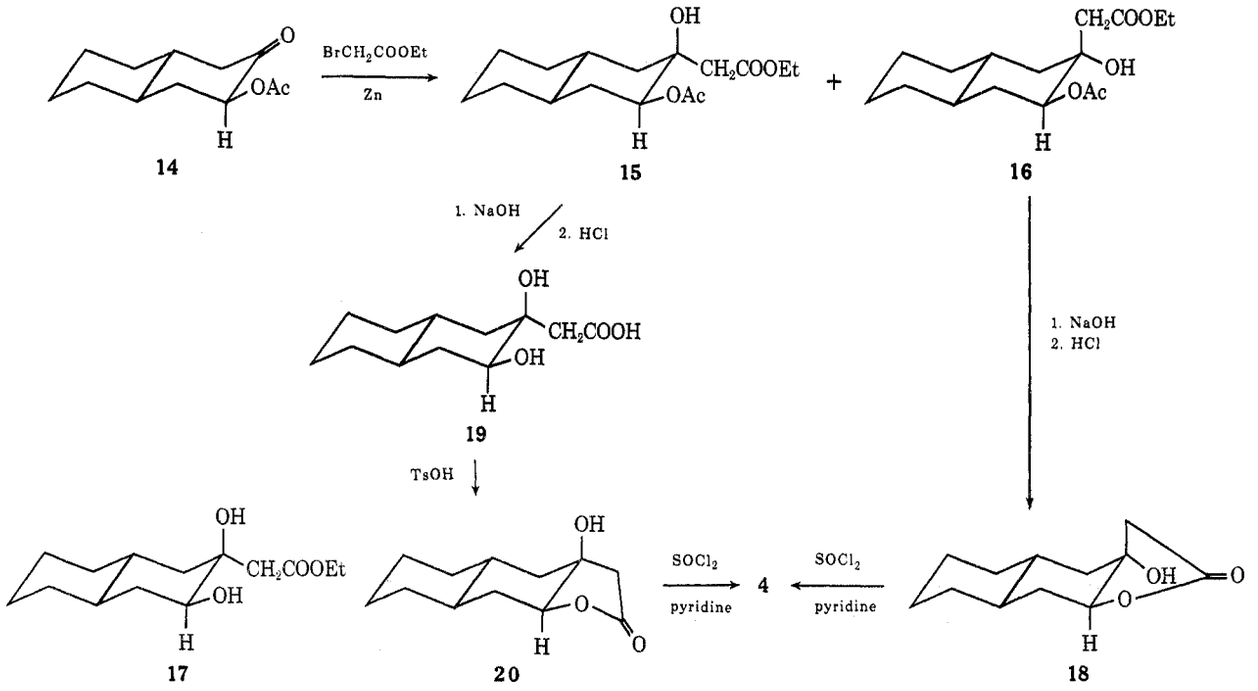
The Reformatsky product **16** on base hydrolysis and acidification gave the hydroxy lactone **18**. Treatment of **18** with thionyl chloride in pyridine produced the butenolide **4**. Base hydrolysis and acidification of **15** yielded an insoluble acid **19**. The acid **19** could be

(12) D. R. Dyer, "Applications of Absorption Spectroscopy of Organic Compounds," Prentice-Hall, Englewood Cliffs, N. J., 1965.

(13) M. W. Rathke and A. Lindert, *J. Org. Chem.*, **35**, 3966 (1970).

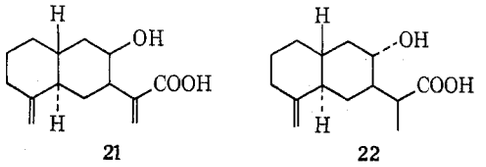
(14) R. J. Ouellette, *J. Amer. Chem. Soc.*, **86**, 4378 (1964).

SCHEME II



converted to hydroxy lactone **20** by refluxing in benzene with a trace of *p*-toluenesulfonic acid. The hydroxy lactone **20** was converted to butenolide **4** by treatment with thionyl chloride in pyridine.

The assigned stereochemistry of the two hydroxy lactones **18** and **20** is supported in two ways. First, Minato and Horibe[15] observed that the cis configuration **21** underwent facile lactonization while the trans configuration **22** required heating to the melting point *in vacuo*.[16] A similar order of reactivity was observed for **18** and **20**. The cis configuration **18** lactonized spontaneously while the trans configuration **20** required refluxing benzene with *p*-toluenesulfonic acid. Secondly, the chemical shift of the hydroxyl proton in deuterated dimethyl sulfoxide of **18** was 12 Hz downfield from that of **20**, which is consistent with an equatorial hydroxyl group in **18** and an axial hydroxyl in **20**.[14]



21 22

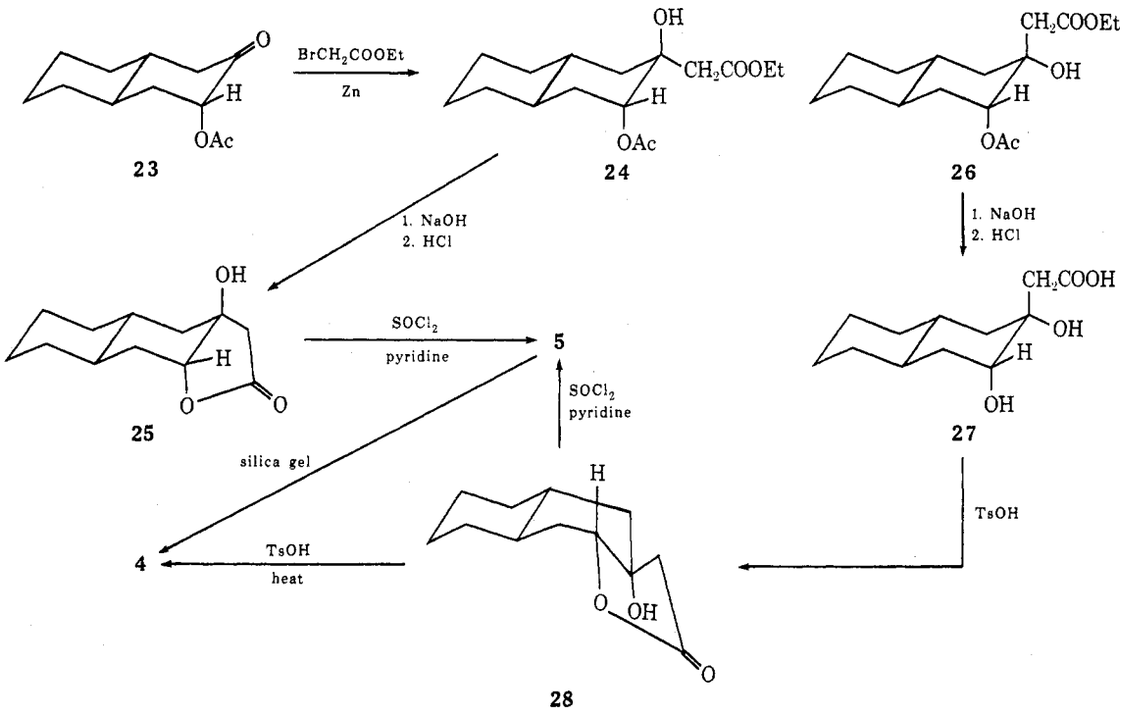
The Reformatsky reaction of 3(*a*)-acetoxy-*trans*-2-decalone (**23**) with ethyl bromoacetate gave a mixture from which **24** could be separated by column chromatography. The proton at C-3 had a peak width at one-half height of 8 Hz (δ 4.80), which is consistent for an equatorial proton and thus an axial acetate. Hydrolysis of **24** in base followed by acidification gave the lactone **25**. The infrared spectrum exhibited a carbonyl stretching at 1780 cm^{-1},[17] while in the nmr the C-3 proton had a peak width of 6 Hz. Both pieces of data support the assigned structure. Treatment of **25** with pyridine and thionyl chloride gave **5** (Scheme III). The infrared spectrum of **5** exhibited carbonyl stretching at 1778 and 1754 cm^{-1} which correspond to reported values for Δ^1-butenolides.[17] The nmr spectrum of **5** showed the C-3 proton as a triplet (J = 7 Hz) with further fine splitting and the vinyl proton as a multiplet ($W_{1/2}$ = 5 Hz) located at δ 5.72. Observance of the C-3 proton signal as a triplet suggests that the one ring of the decalin has flipped to a twist form. If the C-3 proton is approximately 25° from the 4β proton and thus about 145° from the 4α proton, the predicted coupling constant is about 7 Hz, according to the Karplus rule. The twist confirmation just described to fit the nmr data appears to be the most stable one based on Framework Molecular Models[18] of the system.

The butenolide **5** was chromatographed using preparative layer silica gel plates to purify it for analysis, but the epimerized butenolide **4** was obtained.

Attempts to obtain **26** by chromatography of the Reformatsky reaction mixture were unsuccessful. Therefore, the crude reaction mixture was hydrolyzed in base and then acidified. The acid solution was extracted with chloroform to give **25**. A white solid which was not chloroform soluble was filtered and found to be the acid **27**. The axial nature of the C-3 hydroxy group was shown by nmr spectroscopy. The C-3 proton had a peak width of 6 Hz. When the acid **27** was refluxed in benzene with *p*-toluenesulfonic acid, the hydroxy lactone **28** was obtained. A triplet at δ 4.47 (J = 8 Hz) was assigned to the C-3 proton. The observance of a triplet for the C-3 proton signal indicated that the one ring of the decalin system was in the twist form as observed with **5**. Treatment of **28** with thionyl chloride in pyridine gave the axially fused butenolide **5**. When the acid **27** was accidently heated with *p*-toluenesulfonic acid in the dry state, epimerized butenolide **4** was isolated.

(15) H. Minato and I. Horibe, *Chem. Commun.*, 531 (1965).
(16) K. Naemura and M. Nakazaki, *Tetrahedron Lett.*, 33 (1969).
(17) K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962.
(18) Framework Molecular Models, Prentice-Hall, Englewood Cliffs, N. J.

SCHEME III



The stereochemical assignment at C-2 for **24**, **25**, **26**, and **27** was based on the reactions of these compounds. The lactone **25** must be a cis fused lactone because of the ease with which it underwent lactonization. Since the oxygen of the lactone is axial, the acetic acid portion is equatorial. Further, the decalin moiety of **25** appears to be in the chair–chair conformations, which is only possible with the stereochemistry shown. The acid **27** would not be expected to lactonize spontaneously. The carboxyl and the hydroxyl group which lactonize are trans diaxial and thus one ring of decalin must go to a twist form, as was observed, for this reaction to occur.

Further studies of the stereochemistry of Δ^1-butenolides are currently underway and will be presented in a later paper.

Experimental Section

Infrared spectra were recorded on a Perkin-Elmer Infracord or Perkin-Elmer 457 spectrophotometer. Nuclear magnetic resonance spectra were obtained on a Varian Associates T-60 spectrometer using deuteriochloroform as the solvent unless otherwise specified. Data are reported in parts per million (δ) using TMS as internal standard. Melting points were obtained on a Thomas-Hoover Unimelt and are uncorrected. For column and dry column chromatography, E. Merck silica gel containing 15% water (based on R_f of *p*-dimethylaminoazobenzene with benzene as solvent) was used. E. Merck silica gel plates, 2 mm thick, 20 × 20 cm, were used for preparative thin layer chromatography. Microanalyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo.

2-(3-Keto-*trans*-2-decalyl)acetic Acid.—*trans*-2-Decalone (10.0 g) was allowed to react with pyrrolidine (7.0 g) in benzene (100 ml) to give the enamine which was used without further purification. The enamine was alkylated with ethyl bromoacetate (11.0 g), using the procedure of Stork, *et al.*,[19] followed by hydrolysis of the enamine to give ethyl 2-(3-keto-*trans*-2-decalyl)acetate. The keto ester was hydrolyzed with 5% KOH to give 4.34 g (35% based on *trans*-2-decalone) of 2-(3-keto-*trans*-2-decalyl)-acetic acid: mp 92–94° (lit. mp 75–77°, 91.5–92° upon solidification and remelting);[20] ir 1730, 1710 cm^{-1} (C=O); nmr δ 11.70 (s, 1).

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.51; H, 8.62. Found: C, 68.30; H, 8.79.

2-(3(*e*)-Hydroxy-2-decalylidine)acetic Acid γ-Lactone (4).—2-(3-keto-*trans*-2-decalyl)acetic acid (2.0 g) was heated at reflux with acetic anhydride for 3.5 hr using the procedure of Minato and Nagaski[6] to give 1.78 g of a light brown oil. The oil was chromatographed on silica gel (150 g) using a nylon dry column (2.5 × 54 cm) and developed with benzene. The segment of the column 5 to 23 cm from the origin was extracted with $CHCl_3$ to give 1.32 g (72%) of **4** as white crystals. An analytical sample was obtained by recrystallization from chloroform–hexane: mp 75–76°; ir (CCl_4) 1785, 1750 (C=O), 1650 cm^{-1} (C=C); nmr (CCl_4) δ 4.65 (m, 1, J = 10, 6, 1.5 Hz, C-3 H), 5.55 (t, 1, J = 1.5 Hz, CH=C).

Anal. Calcd for $C_{12}H_{16}O_2$: C, 75.01; H, 8.34. Found: C, 74.92; H, 8.36.

2-(1-Keto-*trans*-2-decalyl)acetic Acid (10).—A mixture of *cis*- and *trans*-1-decalone was epimerized to give 6.8 g (45%) of *trans*-1-decalone (**8**) on distillation, mp 28–31 (lit.[21] mp 31–32°). *trans*-1-Decalone (**8**) (6.5 g) was alkylated with ethyl bromoacetate *via* the pyrrolidine enamine as above to give 3.7 g (41% based on **8**) of **10**: mp 153–154° after recrystallization from benzene; ir ($CHCl_3$) 1710 cm^{-1} (C=O); nmr δ 11.68 (s, 1).

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.51; H, 8.62. Found: C, 68.90; H, 8.82.

2-[1(*e*)-Hydroxy-2-decalylidine]acetic Acid γ-Lactone (6).—The keto acid **10** (1.2 g) was cyclized as above to give 1.2 g of brown oil. The oil was chromatographed using the dry column technique with 1.5 × 50 cm nylon column packed with silical gel (100 g) and developed with $CHCl_3$. The section 10 to 18 cm from the top was extracted with $CHCl_3$–CH_3OH to give 650 mg as a fairly pure sample of **6**. An analytical sample was prepared by chromatography on a preparative thin layer plate developed with chloroforom. The major band was scraped off and extracted with 2% methanol in chloroform. The solvent was removed *in vacuo* and the residue was dissolved in 2 ml of $CHCl_3$ and filtered with a sintered glass funnel. Evaporation of the solvent gave an oil which crystallized on standing: mp 40–41°; ir (neat) 2920, 2850 (CH), 1795, 1745 (C=O), 1645 cm^{-1} (C=C); nmr δ 4.39 (q, 1, J = 8, 1.6 Hz, C-1 H), 5.80 (t, 1, J = 1.6 Hz, CH=C).

(19) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Amer. Chem. Soc.*, **86**, 207 (1963).

(20) W. S. Johnson, V. J. Bauer, J. L. Margrave, M. A. Frisch, L. H. Dreger, and W. N. Hubbard, *ibid.*, **83**, 606 (1961).

(21) C. D. Gutsche and H. H. Peter, *ibid.*, **77**, 5971 (1955).

Anal. Calcd for $C_{12}H_{16}O_2$: C, 75.36; H, 7.91. Found: C, 75.40; H, 8.09.

3(*a*)-Acetoxy-*trans*-2(*a*)-decalol.—A solution of decalin 2,3-oxide (9.4 g) and glacial acetic acid (100 ml) was heated on a steam bath for 4 hr and then allowed to stand overnight at room temperature. The solution was evaporated *in vacuo*. The remaining oil was dissolved in chloroform and the solution was washed with aqueous sodium bicarbonate. The chloroform solution was dried ($MgSO_4$), filtered, and evaporated *in vacuo*, leaving 12.8 g (97%) of a viscous, colorless oil which was oxidized without further purification, nmr δ 2.03 (s, $CH_3C{=}O$), 3.80 (m, $W_{1/2}$ = 9.6 Hz, 2(*e*)H), 4.82 (m, $W_{1/2}$ = 7.2 Hz, 3(*e*)H).

3(*a*)-Acetoxy-*trans*-2-decalone (23).—3(*a*)-Acetoxy-*trans*-2(*a*)-decalol (10.0 g) was oxidized with Jones reagent while cooling in an ice bath. Stirring was continued for 2 hr after addition. Isopropyl alcohol was added and then the solvents were removed *in vacuo*. Water was added to dissolve the inorganic salts and then extracted with chloroform. The chloroform solution was dried ($MgSO_4$), filtered, and evaporated *in vacuo*, leaving 9.3 g (94%) of fairly pure **23**: ir (salts) 1745, 1735 (C=O), 1225 cm^{-1} (CO); nmr δ 2.07 (s, $CH_3C{=}O$), 4.82 (m, $W_{1/2}$ = 7.2 Hz, 3(*e*)H). **23** was analyzed as its 2,4-dinitrophenylhydrazone, mp 189–190° from ethyl acetate–ethanol.

Anal. Calcd for $C_{18}H_{22}N_4O_6$: C, 55.38; H, 5.68; N, 14.35. Found: C, 55.32; H, 5.80; N, 14.39.

3(*e*)-Acetoxy-*trans*-2-decalone (14).—A solution of **23** (1.0 g) and glacial acetate (5 ml containing 2 drops of 48% HBr) was allowed to stand at room temperature for 2 days. The solution was evaporated *in vacuo* to give an oil which was dissolved in chloroform. The chloroform solution was washed with aqueous sodium bicarbonate, dried ($MgSO_4$), and evaporated to give 0.92 g of a yellow oil which crystallized on standing. Recrystallization from hexane gave 0.8 g of **14**: mp 57–58° (lit.[22] mp 64–65°); ir (CCl_4) 1740, 1730 (C=O), 1235 cm^{-1} (CO); nmr (CCl_4) δ 2.07 (s, 3), 5.10 (m, 1, $W_{1/2}$ = 20.4 Hz, C-3 H).

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.55; H, 8.74.

Reformatsky Reaction with 3(*e*)-Acetoxy-*trans*-2-decalone (14).—3(*e*)-Acetoxy-*trans*-2-decalone (**14**) (16.3 g) was allowed to react with ethyl bromoacetate (13.05 g) and zinc (5.1 g of 30–60 mesh without purification) in the presence of trimethyl borate (30 ml) and tetrahydrofuran (30 ml) for 60 hr according to the procedure of Rathke and Lindert.[18] Work-up gave 17.3 g of a yellow oil. The oil was chromatographed on silica gel (400 g) using chloroform as solvent and 10-ml fractions were collected. Fractions 121–310 contained 6.57 g of ethyl 2-[3(*e*)-acetoxy-2(*a*)-hydroxy-2(*e*)-decalyl]acetate (**15**): ir (neat) 3690 (OH), 1735, 1720 (C=O), 1240 cm^{-1} (CO); nmr δ 1.28 (t, 3, J = 7 Hz, CH_3CH_2), 2.07 (s, 3), 2.32 (d, 1, J_{gem} = 15 Hz, CH_2CO), 2.62 (d, 1, J_{gem} = 15 Hz, CH_2CO), 4.17 (q, 2, J = 7 Hz, CH_3CH_2), 4.70 (m, 1, $W_{1/2}$ = 17 Hz, C-3 H); nmr (DMSO) hydroxyl proton is 125.6 Hz downfield from strongest peak in DMSO (0.00985 molar ratio). Fractions 311–370 contained 3.43 g of a mixture of **15** and **16**. Fractions 371–405 contained 3.00 g of ethyl 2-[3(*e*)-acetoxy-2(*e*)-hydroxy-2(*a*)-decalyl]acetate (**16**): ir (neat) 3480 (OH), 1730, 1718 cm^{-1} (C=O); nmr δ 1.30 (t, 3, J = 7 Hz, CH_3CH_2), 2.03 (s, 3), 2.50 (d, 1, J_{gem} = 15 Hz, CH_2CO), 2.87 (d, 1, J_{gem} = 15 Hz, CH_2CO), 4.22 (q, 2, J = 7 Hz, CH_3CH_2), 4.83 (m, 1, $W_{1/2}$ = 18 Hz, C-3 H); nmr (DMSO) hydroxyl proton is 135.0 Hz downfield from strongest peak in DMSO. Fractions 402–430 contained 1.30 g of a mixture of **16** and **17**. Fractions 431–460 contained 90 mg of an oil which crystallized on standing. Recrystallization from hexane gave 80 mg of ethyl 2-[3(*e*), 2(*a*)-dihydroxy-2(*e*)-decalyl]-acetate (**17**) as white crystals: mp 92–93°; ir (CCl_4) 3590, 3510 (OH), 1710 (C=O), 1185 cm^{-1} (CO); nmr (CCl_4) δ 1.28 (t, 3, J = 7 Hz, CH_3CH_2), 2.20 (d, 1, J_{gem} = 15 Hz, CH_2CO), 2.82 (d, 1, J_{gem} = 15 Hz, CH_2CO), 3.17 (q, 1, J = 10, 5 Hz, C-3 H), 4.15 (q, 2, J = 7 Hz, CH_3CH_2).

Anal. Calcd for $C_{14}H_{24}O_4$: C, 65.60; H, 9.44. Found: C, 65.70; H, 9.42.

2-[2(*a*),3(*e*)-Dihydroxy-2(*e*)-decalyl]acetic Acid (19).—The ester **15** (1.32 g) was heated on a steam bath with sodium hydroxide (10%, 20 ml) for 6 hr and allowed to stand for 48 hr. The solid which formed was dissolved on addition of water. The basic solution was extracted with chloroform and the chloroform extracts were discarded. The aqueous solution was acidified with hydrochloric acid (10%) to give a solid which was extracted with three portions of chloroform, although the solid was not very soluble in chloroform. The chloroform solution was dried ($MgSO_4$), filtered, and evaporated to give 402 mg (43%) of **19**, mp 175–177°. An analytical sample was recrystallized from methanol–benzene: mp 176–177°; ir (KBr) 3510, 3460 (OH), 1675 cm^{-1} (C=O).

Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: C, 63.11; H, 8.79.

2-[2(*a*),3(*e*)-Dihydroxy-2(*e*)-decalyl]acetic Acid γ-Lactone (20).—A solution of **19** (60 mg) in benzene (20 ml) containing a trace of *p*-toluenesulfonic acid was heated at reflux for 7 hr. The solvent was removed *in vacuo* to give a white solid, mp 160–162°. Recrystallization from benzene–hexane gave **20**: mp 164–164.5°; ir ($CHCl_3$) 3595, 3430 (OH), 1780 cm^{-1} (C=O); nmr δ 2.52 (s, 2, CH_2CO), 4.05 (q, 1, J = 6, 11 Hz, C-3 H); nmr (DMSO) at 0.012 molar ratio the hydroxyl proton is 150 Hz downfield from the strongest peak of DMSO.

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.42; H, 8.75.

Dehydration of 2-[2(*a*),3(*e*)-Dihydroxy-2(*e*)-decalyl]acetic Acid γ-Lactone (20).—Thionyl chloride (1 ml) was dissolved in pyridine (2 ml) and added to a solution of **20** (90 mg) in pyridine (3 ml). The reaction mixture turned black and became hot. It was then heated on a steam bath for 10 min. The reaction mixture was evaporated *in vacuo*. Water (10 ml) was added to the residue and extracted with three portions of chloroform. The chloroform solution was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give a reddish-brown oil. The nmr spectrum was identical with the spectrum of **4** except for a singlet at δ 1.27 and peaks for pyridine. No further purification was performed.

2-[2(*e*),3(*e*)-Dihydroxy-2(*a*)-decalyl]acetic Acid γ-Lactone (18).—A mixture of **16** (540 mg) and 10% aqueous sodium hydroxide (10 ml) was heated on a steam bath for 1.25 hr, at which time solution was complete. The basic solution was acidified with 10% hydrochloric acid and allowed to stand at room temperature for 2 hr and then extracted with three portions of chloroform (15 ml). The chloroform solution was washed with an aqueous sodium bicarbonate solution, dried ($MgSO_4$), filtered, and evaporated to give 217 mg (57%) of oil which crystallized on standing. Recrystallization from benzene–hexane gave an analytical sample of **18**: mp 88.5–89.5; ir ($CHCl_3$) 3610, 3430 (OH), 1775 cm^{-1} (C=O); nmr δ 2.25 (d, 1, J_{gem} = 17 Hz, CH_2CO), 2.72 (d, 1, J_{gem} = 17 Hz, CH_2CO), 4.30 (m, 1, $W_{1/2}$ = 19 Hz, C-3 H); nmr (DMSO) at 0.0115 molar ratio the hydroxyl proton is 162 Hz downfield from the strongest peak of DMSO.

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.51; H, 8.74.

Dehydration of 2-[2(*e*)-Dihydroxy-2(*a*)-decalyl]acetic Acid γ-Lactone (18).—The procedure for this reaction was identical with that for **20** and 80 mg of **18** was used. Work-up gave an oil (60 mg) which crystallized on standing. The nmr spectrum was identical with the spectrum of **4**. No further purification was performed.

Reformatsky Reaction with 3(*a*)-Acetoxy-*trans*-2-decalone (23).—The reaction was conducted on 16.3 g of **23** using the same procedure as for **14**. Work-up of the reaction gave 18.7 g of yellow oil. The oil (3.77 g) was chromatographed using the dry column technique with silica gel (200 g) in a nylon column (3.5 × 45 cm) and developed with chloroform. The section 6 cm from the bottom and 25 cm long was extracted with chloroform containing 10% methanol. Evaporation of the solvent yielded 1.88 g of ethyl 2-[2(*a*)-hydroxyl-3(*a*)-acetoxy-2(*e*)decalyl]acetate (**24**): ir (neat) 3480 (OH), 1725, 1710 (C=O), 1230 cm^{-1} (CO); nmr (CCl_4) δ 1.23 (t, 3, J = 7 Hz, CH_3CH_2), 2.03 (s, 3), 2.34 (s, 2, CH_2CO), 4.11 (q, 2, J = 7 Hz, CH_3CH_2), 4.80 (m, 1, $W_{1/2}$ = 8 Hz, C-3 H).

2-[2(*a*),3(*a*)-Dihydroxy-2(*e*)-decalyl]acetic Acid γ-Lactone (25).—Ethyl 2-[2(*a*)-hydroxy-3(*a*)-acetoxy-2(*e*)-decalyl]acetate (**24**) (500 mg) was heated at reflux with 10% sodium hydroxide (5 ml) for 1 hr. The basic solution was extracted with chloroform and the chloroform was discarded. The basic solution was acidified with 10% hydrochloric acid and extracted with chloroform. The chloroform was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to give an oil (250 mg) which crystallized. Recrystallization from benzene–hexane gave 175 mg (83%) of a colorless crystal (**25**): mp 113–114°; ir (CCl_4) 3615, 3450 (OH), 1780 (C=O), 1220 cm^{-1} (CO); nmr δ 2.40 (d, 1 J_{gem} = 16 Hz, CH_2CO), 2.75 (d, 1, J_{gem} = 16 Hz, CH_2CO), 4.33 (m, 1, $W_{1/2}$ = 6 Hz, C-3 H).

(22) M. E. Ali and L. N. Owen, *J. Chem. Soc.*, 2111 (1958).

Anal. Calcd for $C_{12}H_{18}O_3$: C, 68.55; H, 8.63. Found: C, 68.49; H, 8.53.

2-[3(*a*)-Hydroxy-2-decalylidine]acetic Acid γ-Lactone (5).—The hydroxy lactone **25** (300 mg) was dissolved in pyridine and a solution of thionyl chloride in pyridine was added. The mixture became hot during the addition and was allowed to stand for 20 min. It was evaporated *in vacuo*. Water was added and extracted with chloroform. The chloroform extract was dried ($MgSO_4$), filtered, and evaporated *in vacuo*, which gave a red oil. The oil was chromatographed on silica gel (40 g) developed with chloroform and 75-ml fractions were collected. Fractions 3 and 4 contained 175 mg of a fairly pure sample of **5**: ir (CCl_4) 1778, 1754 (C=O), 1642 cm^{-1} (C=C); nmr δ 5.72 (m, 1, $W_{1/2}$ = 5 Hz), 5.12 (triplet with further fine splitting, 1, J = 7 Hz, C-3 H). In an attempt to purify the sample for analysis, it was chromatographed twice on preparative thin layer chromatography (Brinkman, silica gel, 20 × 20 cm). The first time it was developed two times with chloroform; the second, three times with 50% benzene–chloroform. This treatment completely epimerized the sample to the equatorial butenolide **4**.

2-[2(*e*),3(*a*)-Dihydroxy-2(*a*)decalyl]acetic Acid (27).—The reaction mixture from the Reformatsky reaction with 3(*a*)-acetoxy-*trans*-2-decalone (**23**) (5.69 g) was hydrolyzed by heating overnight on a steam bath with 10% sodium hydroxide (30 ml). A precipitate formed which was soluble on addition of water. The basic solution was extracted with chloroform and the chloroform extract was discarded. The aqueous solution was acidified with 10% HCl and allowed to stand for 3 hr, during which time a precipitate formed. The aqueous mixture was extracted with chloroform. The chloroform was washed with sodium bicarbonate solution, dried ($MgSO_4$), filtered, and evaporated to give 2.42 g of **25**, mp 108–111°. The acidic aqueous solution from above was filtered to give 530 mg of **27**, mp 109–115°. Recrystallization of **27** from methanol–chloroform did not improve the melting point, which was quite variable. It was then recrystallized from acetone and again the melting point was variable. However, if placed in an oil bath at 113° it melted immediately, but if the bath was 111° the range was 111–115°: ir (KBr) 3400–2500 (broad series of peaks), 1705 cm^{-1} (C=O); nmr (CD_3COCD_3) 2.6 (2, s, CH_2CO), 3.77 (m, 1, $W_{1/2}$ = 6 Hz, C-3 H), 4.33 (m, 3, $W_{1/2}$ = 24 Hz, OH).

Anal. Calcd for $C_{12}H_{20}O_4$: C, 63.14; H, 8.83. Found: C, 63.10; H, 9.03.

Lactonization of 2-[2(*e*),3(*a*)-decalyl]acetic Acid (27).—The acid **27** (30 mg) was heated on a steam bath overnight in benzene containing a trace of *p*-toluenesulfonic acid. Solvent was removed *in vacuo*, leaving 2-[2(*e*),3(*a*)-dihydroxy-2(*a*)-decalyl]acetic acid γ-lactone (**28**) as an oily brown solid: nmr (CD_3COCD_3) δ 2.38 (d, 1, J_{gem} = 16 Hz, CH_2CO), 2.72 (d, 1, J_{gem} = 16 Hz, CH_2CO), 3.37 (m, 1, OH) 4.47 (t, 1, J = 8 Hz, C-3 H). When the reaction was repeated using 200 mg of **27**, the benzene accidentally evaporated. The residue was epimerized butenolide **4**.

Attempts to purify **28** by recrystallization resulted in hydrolysis of the lactone to **27**. Treatment of **28** (30 mg) with pyridine and thionyl chloride according to the procedure for **20** gave a brown oil (20 mg). The nmr spectrum of this oil showed the presence of axially fused butenolide **5**.

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Deoxy Oligonucleotide Synthesis *via* the Triester Method

JOSEPH C. CATLIN*[1a] AND FRIEDRICH CRAMER[1b,c]

Max-Planck-Institut fur Experimentelle Medizin, Abteilung Chemie, 34 Göttingen, Hermann-Rein-Str. 3, Germany

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The β-cyanoethyl β′,β′,β′-trichloroethyl phosphate group is used in the triester method of deoxy oligonucleotide synthesis. The utility of this protecting function, and the triester method, is indicated by the synthesis of a number of deoxy di-, tri-, and tetranucleotides, including dCpdCpdTp, dTpdCpdTp, dTpdCpdTpdCp, and dApdTpdTpdCp. The tetranucleotides were prepared by block condensation from two dinucleotide units.

There are compelling biochemical reasons for the synthesis of oligonucleotides of known sequence. The two general chemical approaches, the diester and the triester methods, differ in that in the first the phosphate groups carry an acidic hydrogen while in the second they are fully esterified and, hence, neutral. The diester method is, at present, the better developed; Khorana, *et al.*, have synthesized a gene for alanine transfer ribonucleic acid by the combination of this method and biochemical procedures.[2] The triester method offers three advantages over the diester method: the product can be rapidly purified by chromatography on silica gel, making large-scale synthesis possible; the yields do not fall rapidly with chain length; and the phosphate backbone, being fully esterified, is not susceptible to attack by the condensating agent during each condensation step. Triester methods of oligonucleotide synthesis have been explored using β,β,β-trichloroethyl,[3,4] phenyl,[5] *o*-chlorophenyl,[6] and β-cyanoethyl[7] as phosphate protecting groups.

During an attempt to synthesize DNA codons *via*

* Department of Biochemistry, Medical University of South Carolina, Charleston, S. C. 29401;

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(2) K. L. Agarwal, H. Büchi, M. H. Caruthers, N. Gupta, H. G. Khorana, K. Kleppe, A. Kumar, E. Ohtsuka, U. L. RajBhandary, J. H. Van de Sande, V. Sgaramella, H. Weber, and T. Yamada, *Nature (London)*, **227**, 27 (1970).

(3) F. Eckstein and I. Rizk, *Chem. Ber.*, **102**, 2362 (1969).

(4) T. Neilson, *Chem. Commun.*, 1139 (1969); T. Neilson and E. S. Werstiuk, *Can. J. Chem.*, **49**, 3004 (1971).

(5) C. B. Reese and R. Saffhill, *Chem. Commun.*, 767 (1968).

(6) J. H. v. Boom, P. M. J. Burgers, G. R. Owen, C. B. Reese, and R. Saffhill, *ibid.*, 869 (1971).

(7) R. L. Letsinger and K. K. Ogilvie, *J. Amer. Chem. Soc.*, **91**, 3350(1969).