Kinetically Controlled Protonation of the Enol from Deoxypicropodophyllin

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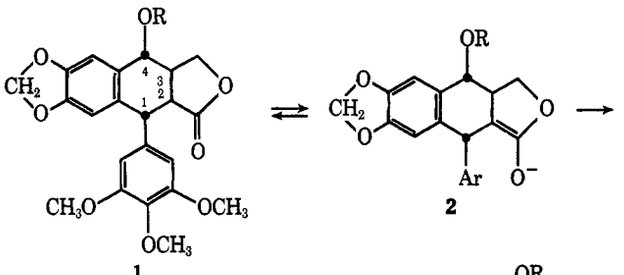
Under a variety of conditions, kinetically controlled protonations of the enolate or enol common to the epimers, deoxypodophyllotoxin and deoxypicropodophyllin, gave mixtures of the two compounds. In one series of experiments, the enolate was prepared by removing a proton with triphenylmethylsodium. In a second series, the enol was formed by decarboxylating 2-carboxydeoxypicropodophyllin. Conversion to the thermodynamically unstable deoxypodophyllotoxin ranged from 13 to 43% and so fell short of reaching the higher conversions anticipated on the basis of postulated transition states and *a priori* arguments. Possible explanations are advanced.

An earlier total synthesis of the anticancer lignan podophyllotoxin (**3**, R = H) calls for enolate formation from a picropodophyllin derivative (**1**, R = tetrahydropyranyl), followed by rate-controlled protonation



1 ⇌ **2** → **3**

of the enolate **2**.[1] The resulting separable mixture of epimers **1** and **3**, although much richer in the thermodynamically unstable podophyllotoxin compound than the equilibrium mixture,[2] still contained more of the thermodynamically preferred picropodophyllin than we had anticipated. The present investigation examined this kind of uphill epimerization with the aim in mind of improving the yield. However, using the closely related enolate **5** from deoxypicropodophyllin (**6**) as substrate, we found under a variety of protonation conditions that the process consistently falls short of giving deoxypodophyllotoxin (**4**) in the proportions expected.[1]

Results

In the work described here, enolate **5** was generated by removing the proton from the 2 position of deoxypicropodophyllin (**6**) or deoxypodophyllotoxin (**4**). Also, the corresponding enol[3] was formed by decarboxylating 2-carboxydeoxypicropodophyllin (**7**).[4] The necessary starting materials were derived from deoxypodophyllotoxin (**4**), which in turn was available by catalytic hydrogenolysis of the podophyllotoxin 4-hydroxy group. Deoxypicropodophyllin (**6**) was prepared by epimerizing deoxypodophyllotoxin under mild basic catalysis[5] while 2-carboxydeoxypicropodophyllin (**7**) was prepared by carbonating enolate **5**.

In one series of experiments, titrating tetrahydrofuran solutions of deoxypodophyllotoxin (**4**) or deoxypicropodophyllin (**6**) with triphenylmethylsodium also in tetrahydrofuran gave the sodium enolate **5**. Introducing a proton source cleanly furnished mixtures of **4** and **6**, which were analyzed by an isotope dilution procedure with the help of deoxypodophyllotoxin (**4**) and deoxypicropodophyllin (**6**) carrying carbon-14 in their 4′-methoxy group. Table I summarizes the yields of deoxypodophyllotoxin with various proton donors. Comparing the results from replicate runs (A-1, B-1, and C-1) showed that the reproducibility was satisfactory, and allowed us to accept the outcome of single runs with more confidence. Our concern about incomplete conversion of substrates to enolate before protonation was allayed when starting with either deoxypicropodophyllin or deoxypodophyllotoxin was found to give the same results (expt A-1 and C-1). Adding enolate to protonating agent or vice versa makes little difference in yields (expt C-1 and C-2). At least with concentrated sulfuric acid, whether the solvent is tetrahydrofuran (A-3) or diethyl ether (E-1) has little effect; the same is true when the enolate is in solution or is present as a suspension.

Sterically hindered protonating agents such as 2,6-di-*tert*-butylphenol (expt B-3) and 2,4,6-trimethylpyridinium (collidinium) hydrochloride (expt D-1) failed to raise the yield of deoxypodophyllotoxin. In this connection, applying the procedure in expt D-3 showed that the sodium 2,6-di-*tert*-butylphenoxide–2,6-di-*tert*-butylphenol system epimerizes deoxypodophyllotoxin to an extent of about 8% in a period as short as 30 sec, a result that was also demonstrated qualitatively in a separate experiment. Experiment C-3 showed that, when a suspension of collidinium hydrochloride in tetrahydrofuran is added to the enolate, the product mixture consists largely of deoxypicropodophyllin and, in fact, probably falls close to the equilibrium mixture.[6] That this result may be a consequence

(1) W. J. Gensler and C. D. Gatsonis, *J. Org. Chem.*, **31**, 4004 (1966).

(2) W. J. Gensler and C. D. Gatsonis, *ibid.*, **31**, 3224 (1966).

(3) *Cf.* Jack Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1962, p 303, especially the contributions of Pederson and of Westheimer. A good review is given also by H. H. Wassermann in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 351.

(4) W. J. Gensler, J. F. X. Judge, and M. V. Leeding, *J. Org. Chem.*, **37**, 1062 (1972).

(5) Acid-catalyzed epimerization is not effective. Deoxypodophyllotoxin in methanol containing some concentrated sulfuric acid does undergo change to other compounds (see Experimental Section), but these do not include deoxypicropodophyllin.

(6) By comparison with the very similar picropodophyllin–podophyllotoxin system,[2] we assume that the equilibrium mixture will contain deoxypicropodophyllin to an extent of *ca.* 97%. Space-filling models (Courtauld) show that the 4-hydroxy group in podophyllotoxin (**3**, R = H) has plenty of room, and that the same is true in the several possible conformations for the more flexible picropodophyllin (**1**, R = H). Replacing the 4-hydroxy group with hydrogen should therefore not effect the equilibrium point substantially. Qualitatively, it has been recognized for some time that deoxypodophyllotoxin can be epimerized to deoxypicropodophyllin in high yield.

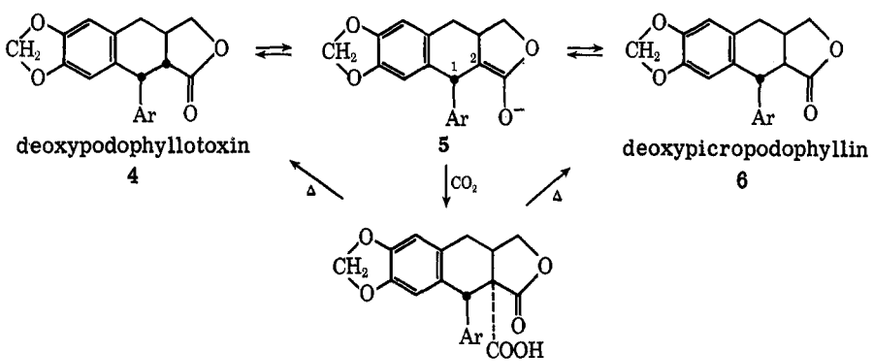


TABLE I

DEOXYPODOPHYLLOTOXIN (4) AND DEOXYPICROPODOPHYLLIN (6) FROM THE PROTONATION OF THEIR COMMON SODIUM ENOLATE (5)

Expt[a]		Protonating agent (amount)	Yield of deoxypodophyllotoxin,[b] %
A[c]	1	Acetic acid (20 ml)	40
	2	Trifluoroacetic acid (20 ml)	39
	3	Concentrated sulfuric acid (2 ml in 50 ml of tetrahydrofuran)	40
B[c]	1	Acetic acid (20 ml)	43
	2	Aqueous sulfuric acid (50 ml of 0.25 *N*)	17
	3	2,6-Di-*tert*-butylphenol (20 g of solid)[d,e]	22[d,e]
C[f]	1	Acetic acid (20 ml)	41
	2	Acetic acid (20 ml)[d]	42[d]
	3	Collidinium hydrochloride (10 g with 80 ml of tetrahydrofuran)[g]	3[g]
D[h]	1	Collidinium hydrochloride[i] with 50 ml of tetrahydrofuran	33[i] (35)[j]
	2	Collidinium hydrochloride plus ^{14}C deoxypodophyllotoxin with 50 ml of tetrahydrofuran[i,i]	*j*
	3	2,6-Di-*tert*-butylphenol (20 g) plus ^{14}C deoxypodophyllotoxin[d,k]	22 (24)[k]
E[l]	1	Concentrated sulfuric acid (1 ml in 20 ml of ether)[l]	38

[a] The capital letter designates a single batch of enolate solution, different portions of which were used as indicated. [b] The yield figure depends on the measured amounts of *both* deoxypodophyllotoxin (5) and deoxypicropodophyllin (7). [c] Enolate was prepared from 2.0 g of deoxypicropodophyllin in 250 ml of tetrahydrofuran. [d] The protonating agent was added to the enolate solution instead of vice versa. [e] To minimize equilibration due to unnecessarily prolonged exposure to the sodium 2,6-di-*tert*-butylphenoxide, aqueous sulfuric acid (50 ml of 0.25 *N*) was added 0.5 min after the phenol protonating agent had been introduced. [f] Enolate was prepared from 1.4 g of deoxypodophyllotoxin in 180 ml of tetrahydrofuran. [g] The collidinium hydrochloride was introduced as a suspension. With the solubility at room temperature determined to be approximately 0.002 *M*, calculation showed that the hydrochloride in solution and thus available for proton delivery corresponded to no more than *ca.* 10% of the initial enolate. Actually, the red color from the small excess of triphenylmethylsodium faded only slowly after mixing the hydrochloride suspension and the enolate solution. [h] Enolate was generated from 1.92 g of deoxypicropodophyllin in 150–200 ml of tetrahydrofuran. [i] The enolate solution was added slowly by drops over a 0.5-hr period to the vigorously stirred suspension of hydrochloride, which had been prepared before by bubbling anhydrous hydrogen chloride into a solution of collidine (0.03 mol) in 50 ml of tetrahydrofuran until the mixture contained approximately 0.02 mol of collidinium hydrochloride and 0.01 mol of collidine. [j] In expt D-2, the radioactivity originally introduced with the protonating agent in the form of labeled deoxypodophyllotoxin (23.53 mg at 28.9 nCi/mg) was recovered as labeled deoxypodophyllotoxin (2.64 nCi/mg) and deoxypicropodophyllin (0.100 nCi/mg). No additional amounts of labeled materials were added for the isotope dilution procedure. From the observed specific activities together with the assay results of experiment D-1, it can be shown that about 7% of the protonation deoxypodophyllotoxin isomerized to deoxypicropodophyllin *after* protonation. Accordingly, the 33% yield observed in this conversion of enolate to deoxypodophyllotoxin should be corrected to 35%. [k] To minimize equilibration due to contact with sodium 2,5-di-*tert*-butylphenoxide, glacial acetic acid was added 0.5 min after the phenol protonating agent had been introduced. Other than the labeled deoxypodophyllotoxin (39.42 mg at 28.9 nCi/mg) added together with the phenol, no additional radioactivity was involved in this experiment. The percentage of protonation deoxypodophyllotoxin recorded in the table is based on the measured activities of recovered deoxypodophyllotoxin (6.60 nCi/mg) and deoxypicropodophyllin (0.222 nCi/mg) as well as on the total amount of enolate used in experiment D-3. The last quantity was obtained by taking the difference between the weight of starting material originally used for enolate formation in the D series[h] and the sum of the weights of protonation deoxypodophyllotoxin and deoxypicropodophyllin recovered in expt D-1 and D-2 as determined by the standard isotope dilution assays. A further result emerging from the data is that 8.5% of the labeled deoxypodophyllotoxin added with the protonating agent is isomerized to deoxypicropodophyllin, so that the apparent 22% protonation conversion to deoxypodophyllotoxin should be corrected to 24%. [l] Enolate was generated from deoxypodophyllotoxin (0.504 mg) in 175 ml of ether by titrating with ethereal triphenylmethylsodium: C. R. Renfrew and C. F. Hauser, "Organic Syntheses," Collect. Vol II, Wiley, New York, N. Y., 1943, p 607; C. R. Hauser and B. E. Hudson, Jr., *Org. React.*, 1, 286 (1942). The ethereal sulfuric acid was added in a single portion to the vigorously stirred, heterogeneous enolate mixture.

of the slow dissolution of solid hydrochloride permitting only a gradual protonation and so providing time for equilibration between the slowly disappearing enolate and its two protonated forms is supported by expt D-1. Here, instead of adding the hydrochloride suspension to the enolate, the enolate was added slowly and with vigorous stirring to the suspension, a simple change that raised the yield of deoxypodophyllotoxin from 3% to over 30%. Where enough collidinium hydrochloride is given a chance to dissolve and always to be available in excess for protonating the enolate, the desired irreversible proton transfer from hydrochloride

to enolate competes successfully with the undesired reversible proton transfer from either **4** or **6** to enolate **5**, so that the complication of equilibration is minimized. Even with this technique, equilibration resulting in a net conversion of deoxypodophyllotoxin to deoxypicropodophyllin is not wholly blocked (*cf.* expt D-1 and D-2).

In the decarboxylation experiments using 2-carboxypicropodophyllin (**7**) as starting material, with the exception of equilibration at higher temperatures, the results are not far different from the direct enolate protonation experiments. In the decarboxylation runs, however, polarimetry instead of isotope dilution was relied on for product analysis. Table II shows that, in the absence of solvent, replicate experiments at 159 and at 170° give satisfactory reproducibility. The pair of results at 199° prove that the presence of added thermodynamically unstable deoxypodophyllotoxin (**4**) affects neither the deoxypodophyllotoxin initially present nor that formed. Also, separate experiments showed that deoxypodophyllotoxin is stable at 200° even in the presence of decarboxylating malonic acid. Temperatures higher than 200° lead to a net conversion of deoxypodophyllotoxin to deoxypicropodophyllin, and so are not applicable for the present purpose.

When decarboxylations are performed with diglyme as solvent (Table III) at temperatures ranging from 90 to 160°, the proportions of deoxypodophyllotoxin vary but little. With tetraglyme as solvent at 95–210° the yields are somewhat lower, but again the variation is small. In the tetraglyme run at 200°, we found that mixing deoxypodophyllotoxin with the starting 2-carboxydeoxypicropodophyllin has no effect on the results. The procedure in the 160° run in diglyme and also in the 210° run in tetraglyme attempted to minimize the time required to bring the sample to the indicated temperatures. However, as the yields show, no difference was detected. The bottom part of Table III records the results of decarboxylation experiments using a variety of solvents. Here, in preliminary pilot experiments, the effect of heating deoxypodophyllotoxin or deoxypicropodophyllin in these solvents with or without decarboxylating malonic acid at various temperatures was found to be minimal. The yields in Table III show clearly that the nature of the solvent has only a secondary

TABLE II

DECARBOXYLATION OF 2-CARBOXYDEOXYPICROPODOPHYLLIN (**7**) IN THE ABSENCE OF SOLVENT

Temp, °C	Time, min	Yield of deoxypodophyllotoxin, %
159	25[a]	27
159	25[a]	30
159	25[a]	36
159	25[a]	34
170	25	35
170	25	33
170	25	32
199	15[b]	30
199	15[b]	28[c]
210	10	22[d]
250	5	9[d]
300	5	4[e]

[a] Bubbling was observed after 5 min when the sample began to melt, and continued for another 10 min. [b] The solid melted with bubbling after 2 min; bubbling continued thereafter for another 5 min. [c] The decarboxylation mixture here consisted of 2-carboxydeoxypicropodophyllin (**7**) (6.890 mg) plus deoxypodophyllotoxin (2.050 mg). The measured composition of the mixture after decarboxylation indicated 46% deoxypodophyllotoxin. Calculation assuming the originally added deoxypodophyllotoxin to persist unchanged shows that 28% of the 2-carboxydeoxypicropodophyllin goes to deoxypodophyllotoxin. This value is close to the 30% obtained in the preceding companion run in which no deoxypodophyllotoxin had been present initially. [d] This figure should be taken as a minimum value, since epimerization of deoxypodophyllotoxin to deoxypicropodophyllin is probably occurring (see text). [e] This value reflects an equilibrium more than a kinetic result (see text).

TABLE III

DECARBOXYLATION OF CARBOXYDEOXYPICROPODOPHYLLIN (**7**) IN SOLUTION

Solvent	Temp, °C	Time, min	Yield of deoxypodophyllotoxin,[a] %
Diglyme	90	30	32
Diglyme	110	30	31
Diglyme	154	10	31
Diglyme	155	10	34
Diglyme	155	4[b]	34[b]
Diglyme	160	2[c]	29[c]
Tetraglyme	95	30	26
Tetraglyme	160	10	22
Tetraglyme	200	4	22
Tetraglyme	200	4	25[d]
Tetraglyme	210	2[c]	22[c,e]
Collidine	150	15	31
Diphenyl ether	190	5	25
Tetralin	190	5	23
Ethylene glycol	190	5	26
Acetophenone	190	5	21
Hexamethyl phosphoramide	190	5	29[e]
Dimethyl sulfoxide[f]	190	5	24[e]
Methyl benzoate[f]	190	5	13

[a] Repeat exposures of the decarboxylation mixtures to the conditions specified did not change the observed rotation, so that decarboxylations could be taken as complete. [b] That the reaction was actually complete even after as short a time as 4 min was shown by the constancy of rotation on further heating. [c] This experiment was performed to see whether the *rate* of temperature increase affected the composition of the decarboxylated product mixture. The sample of 2-carboxydeoxypicropodophyllin (**7**) was dissolved in about 15% of the eventual total volume of solvent. The stirred solution was plunged into the hot oil bath at the same time that the rest of the solvent, preheated to the temperature of the bath, was added. Thin layer chromatographic check showed that no starting material remained after the 2-min reaction period, and that only the expected products were present. The yield of deoxypodophyllotoxin in this experiment is seen to be about the same as from all the others in which the same solvent was used. [d] The starting decarboxylation mixture contained not only 2-carboxydeoxypicropodophyllin (5.863 mg) but also deoxypodophyllotoxin (2.240 mg). The mixture after decarboxylation showed a total content of 47% of deoxypodophyllotoxin. On the assumption that the initially added deoxypodophyllotoxin is not affected in any way, the yield of deoxypodophyllotoxin from the decarboxylation process itself may be calculated as 25%, a value about the same as that obtained in the absence of added deoxypodophyllotoxin. [e] No thin layer chromatography check was made here. [f] Although, as soon as the mixture became hot, it was homogeneous, the starting mixture at room temperature contained undissolved 2-carboxydeoxypicropodophyllin. The solution of products, however, remained in one phase even after returning to room temperature.

effect on the proportion of decarboxylation products formed.

Table IV summarizes the results of decarboxylations

TABLE IV

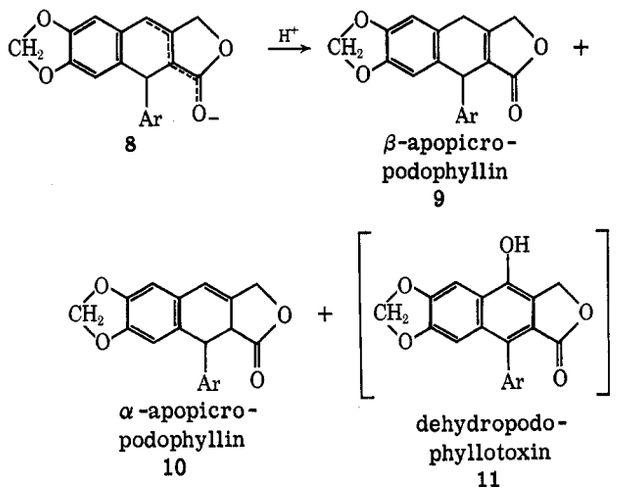
DECARBOXYLATION OF 2-CARBOXYDEOXYPICROPODOPHYLLIN (7) IN THE PRESENCE OF PROTON DONORS[a]

Solvent	Proton donor	Yield of deoxypodophyllotoxin, %
	None	27
	Phenol	27
Tetraglyme	Trichlorophenol	27
	Tribromophenol	27
	2,6-Di-*tert*-butyl-4-methylphenol	27
	None	25
	Pivalic acid	22
Diphenyl ether	Picric acid	25
	Trichlorophenol	24
	p-Toluenesulfonic acid	31

[a] All experiments were carried out over a period of 5 min at 190°.

in the presence of large excesses of various protonating agents with tetraglyme and diphenyl ether as solvents. Again, the resulting proportions of products are remarkably alike even when the proton donors are as different as, for example, phenol in tetraglyme (27% yield of deoxypodophyllotoxin) and *p*-toluenesulfonic acid in diphenyl ether (31%).

As part of this investigation, a small series of protonation experiments was performed with the lithium enolate **8**, which was derived from different though closely related substrates, namely α- and β-apopicropodophyllin (**10** and **9**). With glacial acetic acid con-



8 β-apopicropodophyllin **9**

α-apopicropodophyllin **10** dehydropodophyllotoxin **11**

taining a little concentrated sulfuric acid as the proton source and with air rigorously excluded, the product was a mixture of β-apopicropodophyllin (**9**) and α-apopicropodophyllin (**10**) in the ratio of about 6:1. Although α-apopicropodophyllin (**10**) is unstable with respect to β-apopicropodophyllin (**9**),[7] until the point of equilibrium is known, the question of whether the product mixture is the result of kinetic or equilibrium control cannot be answered. In experiments carried out with only routine care taken to exclude air, substantial amounts of a dehydrogenation product, dehydropodophyllotoxin (**11**), were observed.

(7) A. Robertson and R. B. Waters, *J. Chem. Soc.*, 83 (1933). See also A. W. Schrecker and J. L. Hartwell, *J. Amer. Chem. Soc.*, **74**, 5676 (1952).

Discussion

Our earlier picture of the transition state for protonation had the proton donor positioned above or below the planar enolate portion of the substrate.[1] With this geometry, models suggested convincingly that it would be easier to find room for the donor on the side of the enolate plane *away* from the bulky, quasiaxially disposed 1-trimethoxyphenyl group than on the same side, cis to this group. Accordingly, kinetically controlled protonation was expected to give more deoxypodophyllotoxin than deoxypicropodophyllin. The facts have not borne out these *a priori* arguments. Thus, although the protonations gave deoxypodophyllotoxin in amounts considerably more than in the equilibrium mixture,[6] in no case did the proportions go as high as 1:1. Variation in conditions, *e.g.*, solvent, acid strength of the proton source as well as its type and bulk, reaction temperature, and substrate either as enol or enolate, had relatively minor effects. Generally falling in the 20–40% range, the yields of deoxypodophyllotoxin appear more noteworthy in their similarity than in their difference, and, in terms of kinetic activation parameters, they point to very small energy differences for formation of the two products. The lowest uncomplicated conversion to deoxypodophyllotoxin was 13% (decarboxylation of 2-carboxydeoxypicropodophyllin dissolved in methyl benzoate); the highest conversion was 43% (quenching the sodium enolate with acetic acid). This range corresponds to a difference in Gibbs energy of activation of 1.8–0.2 kcal/mol, with the transition state for the picropodophyllin configuration always at the lower energy level.

The data in Table III allow an estimate of the difference in Arrhenius energies of activation, $\Delta E_a = E_{a(\text{deoxypodo})} - E_{a(\text{deoxypicro})}$. In diglyme solvent at decarboxylation temperatures ranging from 90 to 160°, the yields of deoxypodophyllotoxin average 32 ± 1% (uncertainty given as the mean of deviations from the mean); in tetraglyme solvent at temperatures of 95–210°, the yields, although lower (23 ± 2%), again show the same lack of variation. No trend is obvious in either series. Using these numbers and assigning an arbitrary value of +2 kcal/mol for the difference ΔE_a, in Arrhenius energies, the observed 32% yield of deoxypodophyllotoxin in diglyme at 90° would be expected to increase to 42% at 160° (*cf.* Table V). If, oppositely, ΔE_a is taken as −2 kcal/mol, the 32% yield would have to drop to 23%. In the tetraglyme series, with the same assigned values for ΔE_a, the 23% yield at 95° would have to change to either 36 or 14% at 210°. Since the uncertainty in the experimental values is in the order of 1–2%, these predicted changes of about 10% should be easily detectable, but, as reference to Table III will show, they are not. Accordingly, ΔE_a must be quite small, well within the arbitrary limits of ±2 kcal/mol and possibly close to zero.

In seeking to rationalize these results, we were led to reexamine the basis for choosing our model of the protonation transition state as we did. It developed that the earlier views are far from unanimous in their picture

TABLE V

CALCULATED EFFECT OF TEMPERATURE ON YIELD OF DEOXYPODOPHYLLOTOXIN IN THE DECARBOXYLATION OF 2-CARBOXYDEOXYPICROPODOPHYLLIN

Solvent	ΔE_a[a]	Temp, °C	Calcd yield of deoxypodophyllotoxin, %
Diglyme	+2 kcal/mol	90	[32 kcal/mol][b]
		160	42
	−2	90	[32][b]
		160	23
Tetraglyme	+2	95	[23][b]
		210	36
	−2	95	[23][b]
		210	14

[a] Arbitrarily assumed difference, ΔE_a, in Arrhenius activation energies for the processes leading, respectively, to deoxypodophyllotoxin and deoxypicropodophyllin. [b] Observed value.

of the transition state for protonization or enolization,[8] and that a single generalization for systems involving different enolates, protonating agents, solvents, etc., is not warranted. If in our case, the transition state for development of the picropodophyllin configuration were in fact somewhat removed from the planar starting material, or in other words had moved along the reaction coordinate with development of appreciable off-planar sp^3 geometry of the product, the steric influence of the bulky trimethoxyphenyl group would be weakened,[1] and with it so would the essence of our original argument. The intramolecular energies of the two epimeric products could then play a more determining role in the relative rates of protonation than the intermolecular steric interactions, and, as we actually observed, greater proportions of the thermodynamically more stable picropodophyllin form would emerge.[9] Interestingly, the same kind of interpretation could serve to explain formation of only one stereoisomer, 2-carboxydeoxypicropodophyllin, in the carbonation of enolate **5** as well as formation of only the corresponding ester in the reaction of the same enolate **5** with methyl chloroformate.[4]

From the preparative point of view, in the synthesis of physiologically active though thermodynamically unstable podophyllotoxin compounds from the corresponding picropodophyllin stereoisomers, we have as yet not found a way to improve the key epimerization step. The process, while remaining quite practical and furnishing a quantitative *corrected* yield, still gives only a modest conversion. From the theoretical point of view, the need for caution is reinforced in assuming a near-planar transition state for enolizations or ketonizations, especially where steric effects are appreciable.

(8) *Cf.*, *inter alia*, H. E. Zimmerman in P. de Mayo, "Molecular Rearrangements," Part 1, Interscience, New York, N. Y., 1963, on the "Stereochemistry of Carbanion and Related Reactions," p 352; also F. Johnson, *Chem. Rev.*, **68**, 398 (1968). An overview is given by F. G. Bordwell and K. C. Yee, *J. Amer. Chem. Soc.*, **92**, 5939 (1970). Also see G. S. Hammond, *ibid.*, **77**, 334 (1955); C. G. Swain and A. S. Rosenberg, *ibid.*, **83**, 2154 (1961); C. G. Swain and E. R. Thornton, *ibid.*, **84**, 817 (1962); S. K. Malhotra and H. J. Ringold, *ibid.*, **85**, 1538 (1962); **86**, 1997 (1964); H. Schechter, M. J. Collins, R. Dessy, Y. Okuzumi, and A. Chen, *ibid.*, **84**, 2905 (1962); J. Hine, J. G. Houston, J. H. Jensen, and J. Mulders, *ibid.*, **87**, 5050 (1965); J. Fishman, *J. Org. Chem.*, **31**, 520 (1966).

(9) Johnson[8] prefers another interpretation, namely, that enolate **5** is itself not planar, so that the difference in intermolecular steric hindrance becomes of secondary importance no matter where the transition state is found.

Experimental Section

General.—The general practice described before[1,2,4] was followed here.

Absence of Acid-Catalyzed Epimerization of Deoxypodophyllotoxin (4) to Deoxypicropodophyllin (6).—A solution of deoxypodophyllotoxin[4] (0.2 g) and concentrated sulfuric acid (0.7 ml) in 10 ml of anhydrous methanol was allowed to stand at room temperature for 1 day. Thin layer chromatography (ether–methylene chloride, 6:1) showed a spot at R_f 0.73, corresponding to deoxypodophyllotoxin, plus spots at R_f 0.23 and 0.0. After the solution had been allowed to stand for another day, the same three spots persisted, with the R_f 0.23 spot now dominant. No spot developed at R_f 0.52, where deoxypicropodophyllin appeared in a control run on the same plate. The R_f 0.0 material might be the opened deoxypodophyllic acid, while the R_f 0.23 material is probably methyl deoxypodophyllate.[10]

Labeled Deoxypodophyllotoxin and Deoxypicropodophyllin.—The 4-hydroxy group of podophyllotoxin carrying ^{14}C in its center methoxy group[2] was removed by hydrogenolysis essentially according to the hydrogen-bubbling method reported before.[4] The hydrogenolysis product, diluted with unlabeled deoxypodophyllotoxin, was purified and the colorless needles were finally dried at 100° (0.1 min) over phosphorus pentoxide. This product melted at 165–166° either with or without admixture of authentic deoxypodophyllotoxin, showed a single spot on thin layer chromatography (R_f 0.75 with ether–methylene chloride, 6:1), and was radioactive to the extent of 28.9 nCi/mg.

Labeled deoxypodophyllotoxin was epimerized to labeled deoxypicropodophyllin essentially according to the directions for the unlabeled material.[4] After a 2-day drying period *in vacuo* at 100° over phosphorus pentoxide, the recrystallized white needles (84%) showed mp 170.5–171.5°, gave a single spot on thin layer chromatography (R_f 0.54 with the same solvent as above), and contained 28.2 nCi/mg of ^{14}C.

Enolate Formation, Protonation, and Analysis.—When a red solution of triphenylmethylsodium in tetrahydrofuran[1] was added dropwise to a stirred solution of deoxypodophyllotoxin (or deoxypicropodophyllin) in the same solvent, the red color is discharged quickly, so that the process may be described as a titration. Also, since the time for disappearance of the red color from 1 drop of reagent after a molar proportion has been introduced goes up abruptly, development of a "permanent" red can be taken as a rough end point. The fact that the volume of standardized reagent added in order to reach this end point routinely compared well with the molar amount required bolsters our assumption that the substrate is completely converted to enolate. In any case, a small excess of reagent generally was added past the end point to ensure conversion. With tetrahydrofuran a solvent, the reagent, substrates, and enolate all gave homogeneous solutions. In the few experiments in which ether was the solvent, we noted that homogeneous triphenylmethylsodium in ether reacted rapidly with homogeneous deoxypodophyllotoxin in ether to give a suspension. The red color was discharged relatively slowly when deoxypicropodophyllin, which formed a suspension in ether, was taken as substrate.

To provide more significant comparisons, it was important to be able to protonate portions of the very same batch of enolate with different protonating agents, and for this purpose a manifold gently sloped downward and provided with several stopcocks was sealed to the bottom of the titration flask. Vessels containing carefully weighed quantities of protonating agent were provided with a magnetic stirring bar and were fitted to the stopcocks through ground glass joints. Where needed, pure solvent that had been distilled over lithium aluminum hydride and condensed directly into the still empty titration flask was distributed through the manifold to the several protonation flasks, where homogeneous solutions resulted. Deoxypodophyllotoxin or deoxypicropodophyllin was then added to the titration flask, more solvent was condensed in as desired, and the titration with triphenylmethylsodium was performed as described above. Portions of the enolate solution were added rapidly through the manifold to each of the well-stirred proton sources, which were always in excess. The temperature was held variously at 0–30°. Where desired, the last portion of enolate solution was retained in the titration flask, so that a protonating agent could be added to the enolate instead of vice versa.

(10) *Cf.* J. Renz, M. Kuhn, and A. von Wartburg, *Justus Liebigs Ann. Chem.*, **681**, 207 (1965).

After enolate and proton source were combined, standard chloroform solutions of radioactive deoxypodophyllotoxin and deoxypicropodophyllin were added, and the mixture was stirred further. Water, sometimes with acid, was added, and the aqueous system was concentrated under reduced pressure at temperatures no higher than 50°. The concentrated mixture, containing much precipitate, was extracted with three 60-ml portions of chloroform, and the combined extracts were washed twice with water before drying with sodium sulfate and stripping volatiles off. In some runs, the chloroform extract was also shaken with 3% aqueous bicarbonate. Thin layer chromatography showed that the protonation was clean cut, with the only products being the two expected isomers. Also, control experiments showed that deoxypodophyllotoxin did not epimerize at any stage between protonation and isolation of pure products.

The separation of deoxypodophyllotoxin and deoxypicropodophyllin was accomplished by column chromatography and crystallization. The mixture in 5 ml of chloroform was placed on a 20 × 2.6 cm column of neutral alumina. Elution with 200 ml of chloroform–ether (4:1), 100 ml of ether, ether–methanol (49:1), and finally 250 ml of ether–methanol (1:1) gave various fractions. As shown by thin layer chromatographic monitoring, the 49:1 ether–methanol contained the highest concentration of deoxypodophyllotoxin, while the 1:1 ether–methanol removed most of the deoxypicropodophyllin. The deoxypodophyllotoxin-rich fractions were freed of solvent and then recrystallized from methanol; the deoxypicropodophyllin-rich fractions were recrystallized from aqueous ethanol. In a qualitative though representative run, quenching the enolate with 2,4,6-trimethylbenzoic acid gave deoxypodophyllotoxin (*ca.* 40%), which after two crystallizations showed mp 165–167° and $[\alpha]_D$ −115° (*c* 1, $CHCl_3$) as well as deoxypicropodophyllin, which after two crystallizations showed mp 170–171° and $[\alpha]_D$ +48° (*c* 0.5, C_2H_5OH). In the quantitative runs, as soon as the thin layer chromatography results together with constancy in melting point indicated homogeneity, a sample was retained, which, together with a sample from one additional crystallization, was submitted for radioactivity assay.

To exemplify the results obtained, a sample set of data from an experiment in which the starting substrate was deoxypicropodophyllin and the protonating agent was acetic acid is given here as follows. In the isotope dilution procedure, the weight of radioactive materials introduced was 40.04 mg of deoxypodophyllotoxin (28.9 nCi/mg) plus 33.968 mg of deoxypicropodophyllin (28.2 nCi/mg). The homogeneous protonation products from consecutive crystallizations showed the following activities: deoxypodophyllotoxin, 4.821 and 4.803 nCi/mg[11] (mean, 4.812); deoxypicropodophyllin, 2.944 and 2.963 nCi/mg (mean 2.954). Accordingly, the deoxypodophyllotoxin protonation product weighed [(28.9 − 4.81) = 4.81] × 40.04 = 200 mg, and the deoxypicropodophyllin protonation product correspondingly weighed 290 mg. Thus acetic acid as a proton donor gave rise to a mixture containing 41% of the less stable deoxypodophyllotoxin. In this particular experiment, the same batch of enolate was used in three portions with different protonating agents, each product mixture being analyzed separately. The sum of the weights of the protonation products came to 1.421 g, so that the total recovery checked well with the original weight of deoxypicropodophyllin (1.417 g) taken as starting substrate. The same kind of recovery was noted in other runs.

Table I summarizes the results from the various experiments.

Epimerization of Deoxypodophyllotoxin (4) to Deoxypicropodophyllin (6) with Sodium 2,6-Di-*tert*-butylphenoxide.—With careful exclusion of moisture, 100 mg (0.25 mmol) of deoxypodophyllotoxin was added to a mixture of 5 g (24 mmol) of 2,6-di-*tert*-butylphenol plus 2.5 ml (0.27 mmol) of 0.11 *N* triphenylmethylsodium in tetrahydrofuran in 10–15 ml of the same solvent. Small aliquots of the faintly yellow solution were removed at 1-min intervals, quenched with glacial acetic acid, and analyzed directly by thin layer chromatography (ether–CH_2Cl_2, 6:1). After 1 min, the initial single-spot deoxypodophyllotoxin showed a faint second spot whose R_f value corresponded to deoxypicropodophyllin; after 3 min the spot was more pronounced; and after 5 min the deoxypodo and deoxypicro spots were about the same in appearance.

Polarimetric Assay of Mixtures of Deoxypodophyllotoxin (4) and Deoxypicropodophyllin (6).—Optical rotation measurements of known mixtures of deoxypodophyllotoxin, with $[\alpha]_{589}^{20}$ −112° (*c* 1.0, $CHCl_3$), and deoxypicropodophyllin, with $[\alpha]_{589}^{20}$ +31° (*c* 1.0, $CHCl_3$), showed that the rotation is directly proportional to percentage composition. The determinations, made initially at the sodium line on a Faraday electro-optic effect polarimeter, were subsequently made more conveniently with a Cary recording spectropolarimeter. At 420 nm, deoxypodophyllotoxin shows $[\alpha]_{420}^{20}$ −280° (*c* 0.20, $CHCl_3$) and deoxypicropodophyllin shows $[\alpha]_{420}^{20}$ +100° (*c* 0.20, $CHCl_3$), so that at 420 nm the difference in specific rotations is more than double that at 589 nm. Also, the slopes of the ORD curves at 420 nm were still small. Just as at 589 nm, the rotation of mixtures at 420 nm varied linearly with composition. When decarboxylations were performed in a solvent, the same solvent was used in the rotation measurements as in the decarboxylations. Linearity for the rotation–composition relation was demonstrated with diglyme, tetraglyme, and collidine; linearity was assumed for the remaining solvents.

The uncertainty in specific rotation values attributable to uncertainty in the rotation readings was estimated to range over $[\alpha] = \pm 0.2°$, and so was negligible. A series of four replications of decarboxylation experiments at 159°, by giving yields of deoxypodophyllotoxin varying from 27 to 36% (Table II), suggested that the decarboxylation results can be uncertain to an extent of up to 28% (maximum range of percentage yields divided by the mean) or, from a different viewpoint, up to 10% (mean deviation from the mean divided by the mean). Replicate experiments at 170° gave more reproducible results (Table II), with the same kind of uncertainties estimated at 10 and 3%, respectively.

Decarboxylation of 2-Carboxydeoxypicropodophyllin (7) in the Absence of Solvent.—A 4-in. test tube containing an accurately weighed sample of 2-carboxydeoxypicropodophyllin (8.116 mg) was evacuated and sealed. The glass had been cleaned by soaking it first in hot nitric–sulfuric acid and then in alkaline detergent. After repeated rinsings with distilled water, the tubes were dried at 110°. On placing the sealed tube in an oil bath at 158–160°, no change was seen for about 5 min. Thereafter, over the next 10 min, the material melted and bubbled. After a total heating time of 25 min, the tube was thoroughly cleaned and carefully opened, and the tan glassy contents were dissolved quantitatively in 3.65 ml of chloroform, *i.e.*, in a volume calculated on the basis of quantitative decarboxylation to furnish a solution containing 2 mg/ml. In this particular experiment, dilution was based on a calculated quantitative yield of 7.308 mg; the actual yield as determined by weight difference was 7.410 mg, and so was within 1.4% of the expected. A similar weight check in the other runs showed that the discrepancy between the actual and the calculated weight loss generally was no more than 1%. Thin layer chromatographic analysis of the chloroform solution (CCl_4–ether, 4:1) confirmed that the product consisted only of deoxypodophyllotoxin (4) and deoxypicropodophyllin (6). Polarimetric assay showed that the mixture contained 27% of deoxypodophyllotoxin.

Table II summarizes the results of a series of such decarboxylation experiments. The lowest temperature used was 159°; little decomposition was seen when 2-carboxydeoxypicropodophyllin in quantities greater than capillary samples was exposed to temperatures below 159° for periods of up to 0.5 hr.

A preliminary series of experiments was performed in order to eliminate the possibility that deoxypodophyllotoxin and deoxypicropodophyllin would interconvert after their formation during the heating period. As shown qualitatively by thin layer chromatography and quantitatively by polarimetric assay, deoxypodophyllotoxin alone is unchanged after heating for 35 min at 160° or for 15 min at 250°; deoxypicropodophyllin is similarly stable after heating for 15 min at 250°, or for 10 min at 300°. With the possible catalytic effect of the carboxy group in mind, several experiments were run on mixtures of deoxypodophyllotoxin as well as of deoxypicropodophyllin with about 15% by weight of malonic acid. Rough titration curves taken in 1:1 water–tetrahydrofuran showed that malonic acid (K_A *ca.* 14 × 10^{-5}) is a slightly stronger acid in a water system than the substrate, 2-carboxydeoxypicropodophyllin (K_A *ca.* 2.2 × 10^{-5}). Deoxypodophyllotoxin in the presence of malonic acid (or the

(11) In the counts serving as the basis for the specific activity values, differences between observed counts for two consecutively recrystallized samples generally came to less than 0.5%—only once as high as 1%—of the mean of the two counts. Accordingly, radioactivity purity as well as count reproducibility appeared satisfactory, and uncertainty entering from the radioactivity determinations could be neglected.

acetic acid derived from it), although stable for 35 min at 160° or 15 min at 200°, shows signs of epimerization (32%) to deoxypicropodophyllin after 15 min at 225°, and is almost completed epimerized (90%) after 15 min at 250°. After 10 min at 300°, deoxypodophyllotoxin, either alone or with malonic acid, epimerizes to an extent of *ca.* 93%. Deoxypicropodophyllin in the presence of malonic acid is unchanged after heating for 10 min at 300°.

Decarboxylation of 2-Carboxydeoxypicropodophyllin (7) in Solution.—To an accurately weighed sample (*ca.* 8 mg) of pure 2-carboxydeoxypicropodophyllin in a 4-in. Pyrex test tube was added a volume of distilled solvent calculated to provide a concentration of 2 mg/ml of decarboxylation product assuming complete reaction. The test tube was connected by rubber tubing to a small toy balloon, and the gas space was flushed thoroughly with argon. With the balloon flacid, the tube was dipped into a hot oil bath, and the homogeneous reaction mixture was stirred with the help of a tiny Teflon-covered magnetic bar for the desired time. The optical rotation of the solution at room temperature was measured without delay and without change of solvent. A routine check showed that no solvent was lost by evaporation in these manipulations. That the reaction had gone to completion was proved by returning the solution to the oil bath, repeating the heating procedure, and remeasuring the rotation. In all cases, the rotation remained constant to within 1%. Thereafter, slow removal of solvent by vacuum evaporation at 40° left a residue, which as shown by thin layer chromatography (CCl_4–ether, 4:1) consisted entirely of the expected deoxypodophyllotoxin (4) and deoxypicropodophyllin (6). Table III summarizes the results.

A test of the possibility that the acid group of 2-carboxydeoxypicropodophyllin might catalyze product interconversion was made by heating solutions of the products at various temperatures in the presence of malonic acid. The molar ratio of malonic acid to either product was at least 2:3. In no case under the conditions of the several decarboxylations was more than 4% conversion (generally 1–2%) of deoxypodophyllotoxin (4) to deoxypicropodophyllin (6) observed, nor more than 1% conversion of deoxypicropodophyllin to deoxypodophyllotoxin.

In the same kind of decarboxylation system, either with or without solvent, both the interval before the sample started to bubble and the period during which bubbles were released became shorter as the reaction temperature was raised. So long as the same temperature was used, these periods in experiments with different solvents, either with or without protonating agents present, varied only slightly.

Decarboxylation of 2-Carboxydeoxypicropodophyllin (7) in the Presence of Added Proton Donors.—Dilute, homogeneous solutions of 2-carboxydeoxypicropodophyllin in redistilled tetraglyme or in redistilled diphenyl ether containing a large excess of protonating agent (10 molar proportions) were heated essentially according to the procedure used with the other solution decarboxylations. The quantities of starting material and solvent were taken so that the final summed concentration of deoxypodophyllotoxin and deoxypicropodophyllin, on the basis of a quantitative decarboxylation, would be 2 mg/ml. The unchanged optical rotation observed on repeating the exposure of each mixture to the decarboxylation temperature, as well as the thin layer chromatographic evidence, was consistent with uncomplicated, complete decarboxylations. Also, separate experiments showed that the two decarboxylation products were stable under the conditions of decarboxylation, and that the presence of proton donors had no effect on the rotation–composition line. Table IV gives the results.

Protonation of the Enolate from α-Apopicropodophyllin (10) or β-Apopicropodophyllin (9).—The lithium enolate was prepared from 126 mg (0.32 mmol) of α-apopicropodophyllin[7,12] onto which 15 ml of tetrahydrofuran had been condensed directly from a distillation over lithium aluminum hydride. By liberal use of argon, special care was exerted throughout this experiment to eliminate air. A 0.176 *N* tetrahydrofuran solution of triphenylmethyllithium[4] (2.0 ml or 0.35 mmol) was introduced into the heterogeneous mixture by dropwise addition from a syringe through a serum cap. The red color from each drop of the reagent at first disappeared quite rapidly, but at the end required at least 5 min to fade. The enolate solution, which was now homogeneous, was taken up in a syringe and injected rapidly under the surface of a vigorously stirred mixture of glacial acetic acid (5.0 ml) containing a little concentrated sulfuric acid (*ca.* 0.8 mmol). The acetic acid had been deoxygenated previously by passage of a stream of argon. Thin layer chromatographic analysis of the reaction mixture (CCl_4–CH_3OH, 10:1) showed the presence of β-apopicropodophyllin (9) as a large spot and α-apopicropodophyllin (10) as a faint spot. No spot corresponding to dehydropodophyllotoxin (11) was detected. The ultraviolet absorption curve of the reaction mixture suitably diluted with acetic acid compared closely with the curve taken with a mixture of authentic α-apopicropodophyllin (14%) and β-apopicropodophyllin (86%). The absence of extraneous materials was further substantiated by noting that the ultraviolet absorption curves for the protonation mixture and for the synthetic mixture changed in a closely parallel way when each solution was treated with a drop of piperidine and kept at 55° for 5 hr in order to isomerize α-apopicropodophyllin to β-apopicropodophyllin.[7]

When a similar procedure was applied to 51.8 mg of β-apopicropodophyllin (9), the results were about the same, although here thin layer chromatography revealed α-apopicropodophyllin in the product mixture more as a bulge on the main β-apopicropodophyllin spot than as a discrete spot. The ultraviolet absorption curve compared closely with that of a mixture of 12% α-apopicropodophyllin and 88% β-apopicropodophyllin.

In another experiment starting with α-apopicropodophyllin (112 mg), evidently air was inadvertently admitted. Also, no sulfuric acid was present in the quenching acetic acid. The residue obtained by stripping volatile material under reduced pressures was separated into three fractions by preparative layer chromatography (CCl_4–CH_3OH, 10:1). The fastest moving band was identified by thin layer chromatography as a mixture of triphenylmethane and triphenylmethyl alcohol. The next band consisted of β-apopicropodophyllin (80 mg, 72%) as shown by ultraviolet, infrared, and thin layer chromatographic comparisons. The slowest moving band (16 mg, 14%) proved to be essentially all dehydropodophyllotoxin[13] (mp >250° dec) as shown by ultraviolet, infrared, and thin layer chromatographic comparisons.

To check the effect of oxygen, the lithium enolate was generated in tetrahydrofuran from 124 mg of α-apopicropodophyllin. Then, instead of quenching in acetic acid, the enolate solution was treated with a stream of tank oxygen for 0.5 hr. Preparative layer chromatography as above furnished 15 mg of β-apopicropodophyllin (9) as well as 36 mg of dehydropodophyllotoxin (11). Trace impurities detected in the dehydropodophyllotoxin were not identified.

Registry No.—**4,** 19186-35-7; **5,** 34825-26-8; **6,** 24150-39-8; **7,** 33369-69-6.

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(12) *Cf.* W. J. Gensler, Q. A. Ahmed, Z. Muljiani, and C. D. Gatsonis, *J. Amer. Chem. Soc.*, **93**, 2515 (1971).

(13) *Cf.* W. J. Gensler, F. Johnson, and A. D. B. Sloan, *ibid.*, **82**, 6074 (1960).