The Structure of Cassaic Acid

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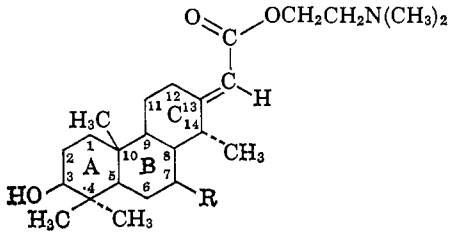
Abstract: The configurations of groups at C-13 and C-14 of the *Erythrophleum* alkaloid cassaine (**1**), and at C-7 of the related alkaloid cassaidine (**2**), are firmly established by direct comparison of *cis* and *trans* isomers at C-13 and and of epimers at both C-7 and C-14.

A study of the *Erythrophleum* alkaloid cassaine (**1**) over a period of 30 years culminated this year in its total synthesis by Turner, Buchardt, Herzog, Morin, Riebel, and Sanders.[1] This synthesis did not, however, define rigorously the *cis vs. trans* nature of the



1, R = O
2, R = β-OH

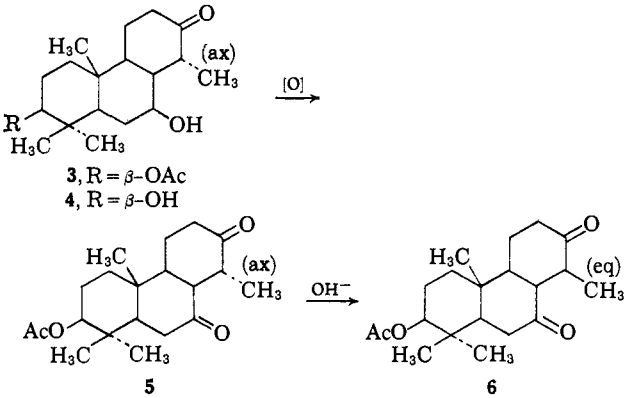
C-13 side chain, the axial (α) *vs.* equatorial (β) character of the C-14 methyl group, or the configuration of the C-7 hydroxyl group in the related alkaloid, cassaidine (**2**). Evidence presented in their work did, however, strongly support the α configuration of the C-14 methyl group.

Hauth, Stauffacher, Niklaus, and Melera[2] studied this configurational problem in detail by nmr spectroscopy using two 2-methylcyclohexanone derivatives as reference models, neither of which possessed exactly the same configuration as that postulated for the natural product. They were able to demonstrate a coupling constant between the C-8 axial hydrogen and the C-14 hydrogen of methyl cassaiate which corresponds to a 45° (or 120°) dihedral angle (Newman projection) between these hydrogens. This observation requires the equatorial configuration for this C-14 hydrogen and, therefore, the C-14 methyl group has the axial ~~co~~nfiguration. A second indication for the axial nature of this methyl group was not valid.[3a]

Hauth and co-workers also present evidence for the *trans* configuration of the side chain in this alkaloid. Footnote 3b presents arguments against the conclusiveness of their evidence.

A direct comparison of the various isomers involved in the structural argument was necessary in order to place the structure of cassaine on a firm basis. In this paper we describe the preparation and comparison of these isomers.

Two compounds, **3** and **6**, reported recently by Turner's group,[1] served as the starting point for our work. The chemical relationship between these compounds is indicated by the oxidation of **3** to **5** and epimerization of **5** to **6**. The configurations at C-7 (not previously proven[4]) and C-14 are correctly represented here. Regardless of actual configurations in-



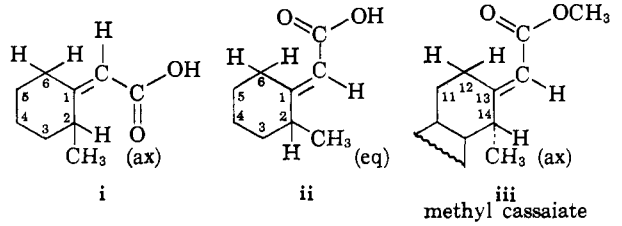
3, R = β-OAc
4, R = β-OH

5 **6**

(1) R. B. Turner, O. Buchardt, E. Herzog, R. B. Morin, A. Riebel, and J. M. Sanders, *J. Am. Chem. Soc.*, **88**, 1766 (1966).

(2) H. Hauth, D. Stauffacher, P. Niklaus, and A. Melera, *Helv. Chim. Acta*, **48**, 1087 (1965).

(3) (a) The models used for these studies were the monocyclic acids i and ii.



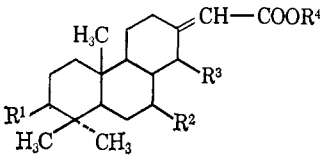
i ii iii
methyl cassaiate

Supportive evidence that the natural product, iii, possesses an axial methyl group derived from the fact that both i and iii showed vinyl hydrogens as doublets resulting, presumably, from their coupling with axial hydrogens at C-6 in the case of i and C-12 in the case of iii. A single peak for the vinyl hydrogen of ii was said to be an unresolved triplet due to coupling of this vinyl hydrogen with axial hydrogens at both C-2 and C-6. We presently report a *trans* ester **15** in which the C-14 methyl group is definitely equatorial. It, therefore, has axial hydrogens at both C-12 and C-14 and should show an unresolved triplet if the above interpretation be correct. It shows a clearly defined doublet. We have no explanation for these doublets. (b) Concerning the *cis vs. trans* nature of the side chain on C-13 of iii, Hauth and co-workers showed that the equatorial C-2 hydrogen of i is deshielded by the carbonyl group of the side chain and appears at 4.1 ppm. The C-2 hydrogen of ii appears normally at 2.0 ppm. It is the C-6 hydrogen of ii which is deshielded by the side chain; it appears at 3.5 ppm. Thus, it seems to be conclusive that i has the *cis* configuration and ii has the *trans* configuration. In attempting to relate methyl cassaiate (iii) with the above models, these workers observed that the C-14 hydrogen of iii appears at 3.05 ppm. Since the signal of this hydrogen is at markedly lower field than the signal of the C-2 hydrogen of the model compound ii (2.0 ppm), the *trans* configuration of iii cannot be deduced from the chemical shift of the signal of the C-14 hydrogen. Such a conclusion is especially unwarranted when these authors fail to show the attendant deshielding of one of the C-12 hydrogens of iii as they have done in the case of compound ii. The difficulty in identifying unequivocally the signal of the C-12 hydrogens in this complex spectrum is, however, fully appreciated.

(4) Without realizing that the configuration of the C-14 methyl group (unknown) determines the course of the reduction of a carbonyl group at C-7, Engel,[5] using sodium borohydride, and V. Arya (*J. Indian Chem. Soc.*, **38**, 419 (1961)), using lithium aluminum hydride, independently concluded that reduction of cassaic acid (the acid derived by hydrolysis of **1**) gives the β configuration to the C-7 hydroxyl group.

(5) B. G. Engel, *Helv. Chim. Acta*, **42**, 1127 (1959).

Table I. Ultraviolet and Optical Rotatory Data on *cis* and *trans* Isomers

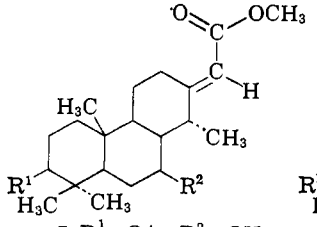


Compound	R^1	R^2	R^3	R^4	Retention[a] time *cis*	Retention[a] time *trans*	Opt rotation $[\alpha]D$, deg *cis*	Opt rotation $[\alpha]D$, deg *trans*	Ultraviolet absorption *cis* mμ	*cis* ε	*trans* mμ	*trans* ε
1	β-OH	=O	α-CH_3	$CH_2CH_2N(CH_3)_2$				−108			224	18,000
2	β-OH	β-OH	α-CH_3	$CH_2CH_2N(CH_3)_2$				−97			224	18,500
7 and **10**	β-OAc	β-OH	α-CH_3	CH_3	1.13	1.30	−14	−90[b]	224	16,500	223	17,900
8 and **11**	β-OH	β-OH	α-CH_3	CH_3	0.89	1.00	−15	−114	224	15,900	224	16,600
9 and **12**	β-OAc	β-OAc	α-CH_3	CH_3	1.10	1.33			222	16,500	221	17,200
15	β-OAc	=O	β-CH_3	CH_3		1.17		−102			223	15,900
20	β-OAc	α-OH	β-CH_3	CH_3				−89			225	17,200
c	β-OAc	=O	α-CH_3	CH_3		1.14					221	18,150

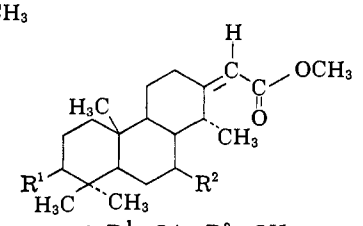
[a] Glpc retention time relative to cholestane using an F & M Model 400 apparatus equipped with a flame ionization detector. Column packing was 3.8% silicone gum SE-30 on 80–100 mesh Diatoport S in a 6-mm o.d. glass tube 4 ft long: column temperature, 240°; injection port temperature, 310°; carrier gas was helium at 60 ml/min. [b] From ref 1. [c] Methyl cassaiate 3-acetate.

volved, it is definite that the hydroxyl and methyl groups of **3** are in the configuration present in cassaine (**1**).[1]

Treatment of hydroxy ketone **3** with trimethylphosphonoacetate in a Wittig reaction gave us methyl cassaidate 3-acetate (**7**) (36%) which was identical in all respects with this ester derived from the natural product **2**. In addition, there was formed 33% of the isomeric substance **10** which we will call methyl isocassaidate 3-acetate.

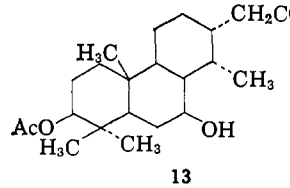


7, $R^1 = OAc; R^2 = OH$
8, $R^1 = R^2 = OH$
9, $R^1 = R^2 = OAc$



10, $R^1 = OAc; R^2 = OH$
11, $R^1 = R^2 = OH$
12, $R^1 = R^2 = OAc$

The fact that these compounds are simply isomeric about the double bond was demonstrated when hydrogenation of both **7** and **10** in the presence of palladium on charcoal produced a common saturated product (**13**). The side chain of **13** was assigned the α configuration on the grounds that the C-14 methyl group hinders approach of the α side of the molecule to the catalyst.



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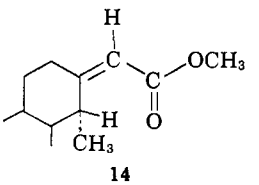

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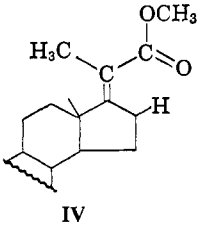
At this point we have further strong evidence that the methyl group at C-14 in this pair of isomers, **7** and **10**, is in the axial (α) configuration. If the C-14 methyl were equatorial, the *cis* isomer **10** would possess such a severe nonbonded interaction between this methyl group and the carbonyl group of the ester as to force an out-of-plane distortion of the chromophore with a resultant major lowering of the ultraviolet extinction coefficient (on the order of 60–70%).[6] Thus, one would expect a major difference between the extinction coefficients of the two isomers. The values given for **7** and **10** in Table I show that such is not the case. The *cis* isomers listed do indeed have slightly lower ε values than do their *trans* counterparts, but this is somehow related to the presence of an oxygen-containing group at C-7.[7] Therefore, the C-14 methyl groups in this pair of isomers, **7** and **10**, must have axial configurations.

When the C-14 methyl group is axial in these α,β-unsaturated esters, the C-14 hydrogen (equatorial) in the *cis* isomer should be in a position for deshielding by the carbonyl group of the side chain (see partial structure **14**). The unnatural isomer of the pair (**10**) did indeed show a hydrogen at 4.45 ppm, occurring in the same position as the multiplet for the C-3 hydrogen which is there by virtue of deshielding by the C-3 acetoxyl group.

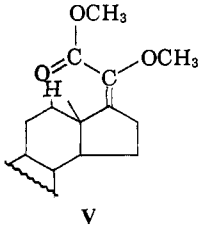
In order to visualize this deshielded C-14 hydrogen more clearly, we prepared the corresponding pair of *cis–trans* isomers with a hydroxyl group on C-3. Trimethylphosphonoacetate reacted with keto-diol **4** to form methyl cassaidate (**8**) (27%) and methyl isocassaidate (**11**) (26%). The identity of the product with the natural configuration was established by acetylation of both diols **8** and **11**, and comparison of the resulting diacetates **9** and **12** with an authentic sample of methyl cassaidate diacetate prepared by acetylation of methyl cassaidate 3-acetate derived from cassaidine (**2**).

Examination of the nmr spectrum of the unnatural isomer **11** now showed the C-14 hydrogen multiplet

(6) N. K. Chandhuri and M. Gut, *J. Am. Chem. Soc.*, **87**, 3737 (1965), report that iv shows ε 9000 and v shows ε 3000, an effect due to out-of-plane distortion of the chromophore. See also W. O. Gotfredsen, W. von Daehne, and S. Vangedal, *Tetrahedron*, **21**, 3505 (1965).



IV

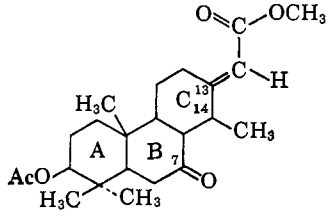


V

(7) To be published.

isolated, and at 4.30 ppm. The identity of this hydrogen was established by the double resonance technique which collapsed the normal C-14 methyl doublet to a singlet. By contrast, the C-14 hydrogen of the natural isomer **8** appears at 2.77 ppm. The strong deshielding of the C-14 hydrogen in the unnatural isomer demands its assignment as the *cis* structure **11** and the natural product must have the *trans* configuration **8**.

Although the needed information was obtained by studying the isomeric pairs **7** and **10**, and **8** and **11**, it was of considerable interest to prepare the *trans* and *cis* forms of a tricyclic ester with the C-14 methyl group in the equatorial configuration as represented by **15** and **16**. Diketone **6**, which has its C-14 methyl group in the unnatural configuration[1] (equatorial), served as our starting material. The reaction of trimethyl phosphonoacetate with diketone **6** was only 40–50% complete. Plate chromatography of the crude reaction



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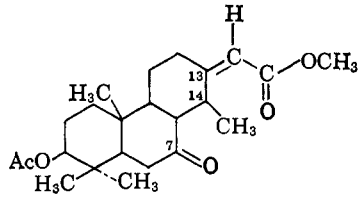

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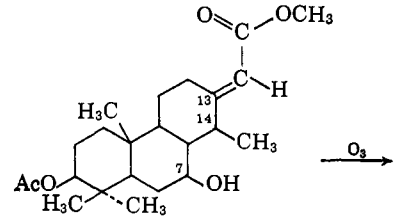
mixture afforded a 27% yield of the *trans* ester **15**.

The other ultraviolet-absorbing band on the chromatoplates should have contained the desired *cis*-equatorial product **16**. In actuality, it contained a mixture of two substances which we could not separate by chromatography or multiple recrystallization. The thrice-recrystallized product melted at 181–183° but was shown by gas–liquid partition chromatography (glpc) to be a 3:7 mixture. From the information at hand, it is impossible to say whether or not isomer **16** was present. Its nmr spectrum indicated that no C-14 methyl doublet had been shifted downfield from the normal position. However, out-of-plane distortion of the side chain could move the C-14 methyl hydrogens out of the deshielding zone of the carbonyl group and prevent this expected effect.

The ultraviolet extinction coefficient of the mixture was low (10,000), but the contribution of each component was not known so this information was not helpful.

There was the possibility that the C-14 methyl group epimerized to the axial configuration under the basic conditions of the Wittig reaction. This phenomenon was observed in a similar stereochemical environment by Szántay, Töke, and Kolonits[8] who also failed to isolate the *cis*-equatorial isomer. One of our products, therefore, might have been methyl cassaiate 3-acetate which indeed has the same glpc retention time as that of the major component of the product mixture. Thin layer chromatography, however, showed that they were definitely different. There is the additional possibility that at least one component of the mixture resulted from reaction of the Wittig reagent at C-7. In essence, we were not able to isolate a *cis*-equatorial isomer.

We stated above that we isolated the *trans* isomer **15** with the methyl group in the equatorial configuration. Proof was required that the Wittig reaction had attacked the C-13 carbonyl group of **6** and not the one at C-7. Reduction of this major product with sodium borohydride produced a mixture of 7-ols, **17** and **20**, with the position of the unreacted carbonyl group now marked with a hydroxyl group. Ozonolysis of each of these 7-ols followed by treatment with base to ensure that the C-14 methyl group was in its more stable configuration furnished two dihydroxy ketones, **19** and **21**, one of which (**19**) was identical with **4**. The formation of **4** requires that the side chain of **17** and likewise of **15** be on C-13 as indicated in their formulas and not on C-7.



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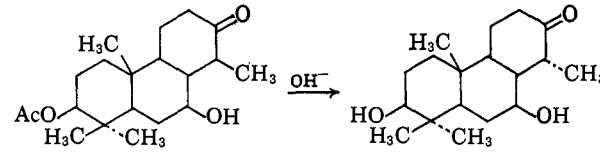
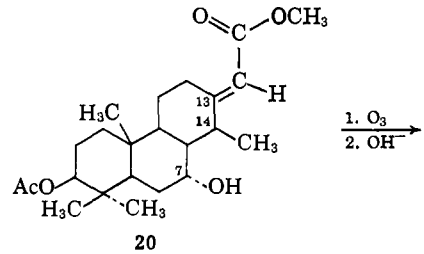


18 **19**



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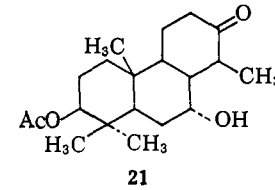

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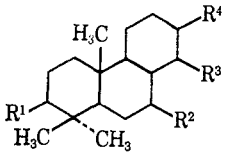
With the side chain of **15** definitely located at C-13, it remains to be shown whether it has the *cis* or *trans* configuration. The nmr spectrum of **15** shows an unaffected C-14 hydrogen at 2.36 ppm (located by double resonance) and a C-14 methyl doublet centered at 1.09 ppm, a value within the range found for this doublet in all *trans* isomers so far studied. (See Table II for a summary of these nmr data.) Since the C-14 methyl group is definitely equatorial and it is not deshielded, there is a good possibility that the side chain of **15** has the *trans* configuration. More definitive evidence is furnished by the ultraviolet extinction coefficient of **15** (ϵ 15,900)

(8) C. Szántay, L. Töke, and P. Kolonits, *J. Org. Chem.*, **31**, 1447 (1966).

Table II. Nmr Methyl Peak Positions in $CDCl_3$



Compound	R^1	R^2	R^3	R^4	Nmr, ppm[c] C-14 methyl		Other methyl groups					
3	β-OAc	β-OH	α-CH_3	=O	1.12	(1.01)	0.88	0.88	0.88	(0.60	0.85	0.85)
5	β-OAc	=O	α-CH_3	=O	1.07	(1.05)	0.85	0.90	1.00	(0.48	0.67	0.72)
6	β-OAc	=O	β-CH_3	=O	1.05	(1.32)	0.88	0.93	1.12	(0.55	0.72	0.72)
7	β-OAc	β-OH	α-CH_3	=CHCOOCH$_3$ (*trans*)	1.06	(0.99)	0.85	0.85	0.88	(0.62	0.87	0.87)
10	β-OAc	β-OH	α-CH_3	=CHCOOCH$_3$ (*cis*)	1.06	(1.06)	0.87	0.88	0.90	(0.62	0.82	0.83)
8	β-OH	β-OH	α-CH_3	=CHCOOCH$_3$ (*trans*)	1.06		0.80	0.80	1.00			
11	β-OH	β-OH	α-CH_3	=CHCOOCH$_3$ (*cis*)	1.06		0.80	0.80	0.98			
15	β-OAc	=O	β-CH_3	=CHCOOCH$_3$ (*trans*)	1.09	(1.13)	0.88	0.92	1.08	(0.62	0.72	0.72)
17	β-OAc	β-OH	β-CH_3	=CHCOOCH$_3$ (*trans*)	1.25		0.72	0.87	0.87			
20	β-OAc	α-OH	β-CH_3	=CHCOOCH$_3$ (*trans*)	1.08	(0.98)	0.78	0.85	0.85	(0.55	0.83	0.83)
a	β-OAc	=O	α-CH_3	=CHCOOCH$_3$ (*trans*)	1.05	(1.05)	0.87	0.92	0.98	(0.53	0.67	0.72)
b	=O	=O	α-CH_3	=CHCOOCH$_2$ (*trans*) $\vert$ $(CH_3)_2N—CH_2$	1.06	(1.00)	1.08	1.08	1.20	(0.55	0.70	0.90)

[a] Methyl cassaiate 3-acetate: L. Ruzicka, G. Dalma, and W. E. Scott, *Helv. Chim. Acta*, **24**, 63 (1941). [b] 3-Dehydrocassaine, ref 9. [c] Values in parentheses were measured in benzene.

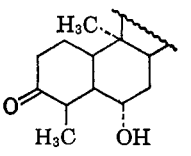
and of its reduction product **20** (ε 17,200), both of which are reasonable for members of the *trans* series and entirely out of the 4000–7000 range predicted for the *cis*-equatorial isomer.

Consideration of the 7-ols, **17** and **20**, reveals a basis for assigning a configuration to the C-7 hydroxyl group of cassaidine (**2**) and hydroxy ketone **3**. These alcohols, **17** and **20**, have a common origin in ketone **15** and, therefore, differ only in being epimeric at C-7. The minor product (22%) can be assigned the β-configuration **17** because the 7β-ol eclipses and thereby deshields the C-14 β-methyl group and causes its nmr doublet to be shifted downfield to 1.25 ppm, the first significant shift of this methyl resonance in the whole series studied (see Table II). Such deshielding effects from eclipsed hydroxyl–methyl interactions are well documented.[9]

The major reduction product (60%) can then be assigned the 7α-configuration **20**, its being epimeric with **17**. This 7α-hydroxy compound shows a normal C-14 methyl resonance position at 1.08 ppm (see Table II). An explanation for the predominance of the 7α-alcohol in this reduction probably lies in inhibition of 7β-ol formation through a developing eclipsing interaction between the C-14 methyl group and the C-7–O-boron complex. This type of "product development control" is well known.[10] In contrast, reduction of cassaine (**1**) gives almost exclusively the 7β-ol.[1,5] Here the C-14 α-methyl group opposes development of the 7α-ol.

An added assurance that the C-14 methyl group was still equatorial (β) in these latter reactions was found in the ozonolysis of **17** and **20**. In each case, a small quantity of diketone **6** (14β-methyl) was isolated as a result of oxidation at C-7. Our experience and that of Turner's group[1] indicates that no epimerization at C-14 occurs under these conditions of ozonolysis.

Turner's group[1] found that optical rotatory dispersion measurements (ORD) on the epimeric diketones **5** and **6** gave strong negative and positive Cotton effects, respectively, in accord with predictions of the octant rule.[11] Since ORD results are sometimes unpredictable where two carbonyl groups are in proximity,[12] we tried to glean information from hydroxy ketone **3** (see rotated partial structure **22** below). This compound shows a positive Cotton effect curve of low amplitude (+7) which matches the amplitude found in the ORD curve of 4β-methyl-5α-cholestan-3-one (**23**) most closely of the models compared[13] (see Table III). It turns out that all of these ORD results are in accord with the correct configuration.



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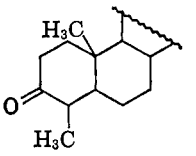

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Table III. Optical Rotatory Dispersion Comparisons

Compound	Amplitude
5α-Cholestan-3-one	+45
2α-Methyl-5α-cholestan-3-one	+62
2β-Methyl-5α-cholestan-3-one	+73
4α-Methyl-5α-cholestan-3-one	>+50
4β-Methyl-5α-cholestan-3-one	+7
Tricyclic ketone **3**	+7

(9) A compilation by N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day Inc., San Francisco, Calif., 1964, pp 19–20, 29–30, illustrates such deshielding of the C-19 methyl protons by 2β-, 4β-, 6β-, and 11β-hydroxyl groups. O. Lindwall, F. Sandberg, R. Thorsen, and T. Norin, *Tetrahedron Letters*, 4203 (1965), report that the 4α-methyl group of erythrophleguine, which also contains a 6α-hydroxyl group, appears at lower field (1.44 ppm) than it does in cassamic acid (1.16 ppm) which does not contain a 6α-hydroxyl group. This same report makes reference to unpublished information to the effect that the 4α-methyl group in 13-epimanool appears at 0.86 ppm whereas 6α-hydroxy-13-epimanool exhibits a 4α-methyl resonance at 1.08 ppm.

(10) W. G. Dauben, E. J. Blanz, Jr., J. Jin, and R. A. Micheli, *J. Am. Chem. Soc.*, **78**, 3752 (1956).

(11) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *ibid.*, **83**, 4013 (1961).

(12) C. Djerassi and W. Closson, *ibid.*, **78**, 3761 (1956).

(13) We greatly appreciate the kindness of Dr. Gloria G. Lyle in making this ORD determination and comparison.

Table II contains a summary of the nmr data obtained from the present study. The normal range for the C-14 methyl group seems to be 1.05–1.12 ppm (in $CDCl_3$). The effect on these peak positions in shifting from chloroform to benzene as a solvent system affords useful information. Connolly and McCrindle[14] discuss chloroform *vs.* benzene-induced changes of chemical shifts with lead references and offer a generalization to the effect that those hydrogens are deshielded which lie on the oxygen side of a plane passing through the carbon of a carbonyl group and perpendicular to the carbon–oxygen direction. In accord with this prediction, the C-14 methyl protons of compound **15** of Table II are seen to be deshielded (Δ = 0.04 ppm) owing to the effect of the C-7 carbonyl group. Compound **6** shows a much greater deshielding effect (Δ = 0.27 ppm), presumably the result of deshielding by carbonyl groups at both C-7 and C-13. Both of these deshielding effects support the assignment of the β (equatorial) configuration to this methyl group.

It should, however, be noted that in the spectrum of compound **20** the chemical shift of the C-14 methyl group changes by −0.1 ppm (deuteriochloroform *vs.* benzene), *e.g.*, even in the absence of a keto group. It seems, therefore, that olefin and/or alcohol and/or ester functions can noticeably contribute to the quoted solvent effect. Without a better understanding of the magnitude and direction of the solvent effects induced by these groups, other established solvent effects (*e.g.*, associated with the keto group) can only be interpreted with great caution if one or more of these groups is also present.

Although it is of no significance to the present structural argument, it is of interest that the solvent change discussed causes *all three* other methyl groups to be shielded substantially if a carbonyl group is at C-7 (compounds **5**, **6**, **15**, methyl cassaiate 3-acetate, and 3-dehydrocassaine). The observed effect on the signals of these three methyl groups is much larger than the effect on the signal of the closer C-14 methyl group. In the rest of the compounds of Table II *only one* of the three methyl peaks is shifted substantially. There is no obvious basis for assignment of these peaks to specific methyl groups.

As noted earlier, whenever a pair of *cis–trans* isomers has been available in the present study, the *trans* isomer shows a greater ultraviolet absorption coefficient than does the *cis* isomer (see Table I). From this same table it can be seen that the specific rotations of the *trans* compounds are significantly more negative than those of the *cis* compounds. The *trans* isomers also show greater glpc retention times and greater R_f values on silica chromatoplates than do their *cis* counterparts.

Experimental Section

General Methods. All optical rotations were measured in chloroform unless noted otherwise. The melting points are corrected. The infrared spectra were recorded on a Perkin-Elmer infrared spectrophotometer, Model 21. KBr disks (0.75%, w/w) were used, or 1% solutions in CS_2, or 10% solutions in $CHCl_3$ in 1-mm or 0.1-mm cells, respectively. The spectra of compounds which were available only in quantities of less than 1 mg were recorded on a Beckman infrared spectrophotometer, Model 7, equipped with a beam condenser. The ultraviolet spectra were recorded on a Cary spectrophotometer, Model 15. Ethanolic solutions (about 0.01 to 0.02 g/l.) in 1-cm cells were used. The nmr spectra were recorded on a Varian A-60 nmr spectrometer. Solutions (10–20%, w/v) in $CDCl_3$ were used, which contained TMS as internal standard (0 ppm). Chemical shifts were measured on precalibrated chart paper. Spirodecoupling experiments were performed on a Varian HA-100 nmr spectrometer.

Methyl Cassaidate 3-Acetate (7) and Methyl Isocassaidate 3-Acetate (10) from 2β,9β-Dihydroxy-8α-methylpodocarpan-7-one 2-Acetate (3). To 48 mg (1.0 mmole) of 50% sodium hydride in oil, which was suspended in 5 ml of dry dimethylformamide, was added a solution of 0.182 g (1.0 mmole) of trimethyl phosphonoacetate in 5 ml of dry dimethylformamide in a fast stream of drops at 5–10° with stirring. The mixture was stirred for 0.75 hr and was then treated with a solution of 168 mg (0.50 mmole) of 2β,9β-dihydroxy-8α-methylpodocarpan-7-one 2-acetate,[1] mp 134–135.5°, in 5 ml of dry dimethylformamide added during 3 min at 20–25°.

The progress of the reaction was followed by thin layer chromatography on silica gel using pure ether for development. The reaction was about 70% complete in 21 hr and perhaps 90% complete in 48 hr.

After standing 48 hr at room temperature, the reaction mixture was diluted with 200 ml of water, and this mixture was extracted twice with ether. The ether extracts were washed twice with saturated salt solution, dried (Na_2SO_4), and concentrated to a residue. Preparative tlc using silica gel and development with ether–pentane (3:2) enabled the isolation of two ultraviolet-absorbing products. The less polar of these products, mp 141–148° (0.07 g, 36%), was methyl cassaidate 3-acetate (7). It was rechromatographed on a silica plate and then recrystallized from 1 ml of boiling ether to which was added pentane with maintenance of volume until a faint cloudiness developed. Cooling gave 63 mg of needles of a dimorphic product which melted at 144–145° when inserted at 130°, but largely melted and resolidified when inserted at 142°. This material proved to be identical with an authentic sample[1] [mixture melting point, infrared curve (CS_2), and tlc] which also showed a double melting point and was observed to crystallize as plates on occasion.

Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.2; H, 9.3.

The more polar product, mp 154–158° (0.065 g, 33%), was **methyl isocassaidate 3-acetate (10)**. It was recrystallized from ether–pentane in the manner described for its isomer above to give 53 mg of material melting at 157–160°. Rechromatography on a silica plate and an additional recrystallization from ether–pentane afforded the analytical sample, mp 159–160°, $[\alpha]^{25}D$ −14°.

Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.2; H, 9.4.

Hydrogenation of Methyl Cassaidate 3-Acetate (7). Palladium-on-charcoal catalyst (10%, 30 mg) was added to a solution of 30 mg of methyl cassaidate 3-acetate, mp 144–145°, in 20 ml of undenatured 95% ethanol, and the mixture was treated with hydrogen under 47 psig for 2.5 hr at room temperature. The mixture was filtered, and the filtrate was concentrated to a residue by warming *in vacuo*. This crystalline residue was recrystallized twice by dissolving it in 1 ml of boiling ether and gradually replacing the ether with pentane with maintenance of volume until crystals began to separate. Thus, 20 mg (66%) of **methyl dihydrocassaidate 3-acetate (13)** was obtained as thin plates, mp 132–134.5°. A third recrystallization raised the melting point to 133.5–135°, $[\alpha]^{25}D$ +12.0, no ultraviolet absorption. Its infrared spectrum (KBr pellet) showed strong carbonyl peaks at 5.76 and 5.89 μ with very weak peaks at 5.66 and 6.02 μ. A solution in CS_2 showed only a strong 5.76-μ carbonyl band.

Anal. Calcd for $C_{23}H_{38}O_5$: C, 70.01; H, 9.71. Found: C, 69.0; H, 9.8.

Hydrogenation of Methyl Isocassaidate 3-Acetate (10). Palladium-on-charcoal catalyst (10%, 20 mg) was added to a solution of 14 mg of methyl isocassaidate 3-acetate, mp 159–160°, in 15 ml of undenatured 95% ethanol, and the mixture was treated with hydrogen under 47 psig for 3.5 hr at room temperature. The reaction mixture was worked up as described in the preceding experiment except that the product was recrystallized three times instead of twice. **Methyl dihydrocassaidate (13)** (7 mg, 50%) was thus obtained, mp 132–134.5°, and undepressed upon admixture with the sample prepared by hydrogenation of methyl cassaidate 3-acetate. The infrared spectra of these two products in CS_2 were superimposable.

2β,9β-Dihydroxy-8α-methylpodocarpan-7-one (4). A solution of 0.40 g (4.0 mmoles) of potassium bicarbonate in 2 ml of water

(14) J. D. Connolly and R. McCrindle, *Chem. Ind.* (London), 379 (1965).

was added to a solution of 0.27 g (0.8 mmole) of 2β,9β-dihydroxy-8α-methylpodocarpan-7-one 2-acetate[1] in 8 ml of methanol, and the resulting mixture was heated under reflux for 8 hr. It was then allowed to stand for 65 hr at room temperature. Dilution of the mixture with 20 ml of water and filtration afforded a solid product which was chromatographed on a 20 × 40 cm plate coated with a 1-mm layer of silica gel. Ether–methanol was used to develop the plate. The eluate from the principal band afforded a solid which was recrystallized from methanol to give 113 mg (48%) of faintly yellow crystals, mp 180–182° and unchanged by recrystallization from acetonitrile; $[\alpha]^{25}D$ −11.5° (1% in MeOH); λ_{max}^{KBr} 2.95–3.05 and 5.88 μ.

Anal. Calcd for $C_{18}H_{30}O_3$: C, 73.43; H, 10.27. Found: C, 73.2; H, 10.2.

Methyl Cassaidate (8) and Methyl Isocassaidate (11) from 2β,9β-Dihydroxy-8α-methylpodocarpan-7-one (4). A solution of 226 mg (1.2 mmoles) of trimethyl phosphonoacetate in 4 ml of dry dimethylformamide was added to a suspension of 45 mg (0.91 mmole) of 50% sodium hydride (in oil) in 5 ml of dry dimethylformamide with stirring at 25°. A transient precipitate formed. After 15 min, 92 mg (0.31 mmole) of 2β,9β-dihydroxy-8α-methylpodocarpan-7-one (**4**) was added. Tlc on samples of the mixture showed that the reaction was about 90% complete in 23 hr. After 46 hr, the mixture was worked up as described for the corresponding 3-acetates (7 and **10**). The plate chromatography was done using ether–pentane (9:1) for development of the plates, three passes of solvent being necessary for adequate band separation.

The two major bands were scraped from the plates and eluted with ether. The less polar band afforded 0.05 g of **methyl cassaidate (8)** which was recrystallized twice by diluting a concentrated solution of it in acetone with pentane. The resulting needle clusters (30 mg, 27%) melted at 160.5–161°; $[\alpha]^{25}D$ −113.8° (1% in EtOH); λ_{max}^{KBr} 2.95, 5.82, 5.88, and 6.10 μ.

Anal. Calcd for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.9; H, 9.9.

The more polar band afforded 0.045 g of **methyl isocassaidate (11)** which was recrystallized in the manner described above to give needles (29 mg, 26%), mp 193–194.5°; $[\alpha]^{25}D$ −15.0° (1% in EtOH); λ_{max}^{KBr} 2.89, 5.82, 5.88, and 6.11 μ; $\lambda_{max}^{CHCl_3}$ singlet carbonyl 5.84 μ.

Anal. Calcd for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 71.8; H, 9.7.

Methyl Cassaidate Diacetate (9) from Natural Sources. Methyl cassaidate 3-acetate (15 mg), prepared from natural cassaine,[1] was acetylated by treatment with 0.6 ml of acetic anhydride and 0.6 ml of pyridine at 100° for 1 hr. The mixture was diluted with water and the product separated with ether. Chromatography of the crude oily product on silica chromatoplates developed with 1:1 ether–pentane furnished a quite pure, yet oily diacetate. It showed $\lambda_{max}^{CS_2}$ 5.77, 5.83, and 8.08 μ, glpc retention time 1.33 relative to cholestane.

Anal. Calcd for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 69.0; H, 8.7.

Methyl Cassaidate Diacetate (9) from Synthetic Methyl Cassaidate (8). Methyl cassaidate (**8**) (10 mg), which had been prepared by the Wittig reaction on tricyclic ketone **4**, was acetylated and purified in the manner described immediately above. The infrared spectrum and glpc retention time of the oily methyl cassaidate diacetate so obtained were identical with those of methyl cassaidate diacetate described above which had been prepared from cassaine. Glpc showed that this product was 98.8% pure and that the remaining 1.2% was the *cis* isomer.

Methyl Isocassaidate Diacetate (12). Acetylation of 10 mg of methyl isocassaidate (**11**) by acetic anhydride in pyridine at 100° for 1.3 hr with work-up and purification as described immediately above furnished a crystalline diacetate. Recrystallization from boiling ether through repeated addition of pentane furnished colorless needles, mp 150–151°; $\lambda_{max}^{CS_2}$ 5.76, 5.82, and 8.08 μ; glpc retention time 1.10 relative to cholestane. The infrared spectrum of this diacetate is definitely different from that of methyl cassaidate diacetate (**9**), and the R_f value on a silica chromatoplate developed with ether–pentane (1:4) is slightly less than that for **9**. This methyl isocassaidate diacetate was 99.2% pure (glpc) and contained 0.5% of methyl cassaidate diacetate.

Anal. Calcd for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 69.0; H, 9.1.

Methyl 14-Epicassaiate 3-Acetate (15). A solution of 182 mg (1 mmole) of trimethyl phosphonoacetate in 4 ml of dry dimethylformamide was added to a suspension of 48 mg (1 mmole) of 50% sodium hydride (in oil) in 5 ml of dry dimethylformamide at 5° with stirring. This mixture was stirred for 1 hr at room temperature and was then treated with 167 mg (0.50 mmole) of 2β-hydroxy-8β-methylpodocarpane-7,9-dione acetate[1] (**6**) at room temperature. The resulting solution was allowed to stand at room temperature for 93 hr. Progress of the reaction was followed by development of samples on silica chromatoplates using 2% MeOH–48% ether–50% pentane for development. No further change was visible during the last portion of the 4-day period. It appears that only about 40–50% reaction occurred.

The mixture was diluted with 200 ml of water and extracted twice with ether. The extracts were washed with brine and concentrated to a residue.

A repeat experiment was done using 54 mg of sodium hydride, 204 mg of trimethyl phosphonoacetate, and 187 mg of the diketone, but this time the reaction mixture was heated at 100° for 1 hr after standing initially for 16 hr. Tlc indicated that the same ratio of products was formed at the elevated temperature. The reaction products were isolated as described above, and the crude mixtures from both experiments were combined.

Separation of the components of the mixture was accomplished on 8 × 16 in. chromatoplates coated with a 1-mm layer of silica gel. Development by two passes of 2% methanol–48% ether–50% pentane gave two heavy bands of ultraviolet-absorbing material. The lead band, R_f about 0.6, was eluted to give 0.11 g (27%) of **methyl 14-epicassaiate 3-acetate**. Recrystallization from acetonitrile afforded 75 mg (18%) of plate clusters, mp 214–217°, $[\alpha]^{25}D$ −101.9°, λ_{max}^{KBr} 5.80 and 5.87 μ (two carbonyl groups superimposed to give 5.80 μ peak). The melting point was unchanged upon recrystallization of the sample.

Anal. Calcd for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.7; H, 8.7.

The trailing ultraviolet-absorbing band, R_f about 0.5, from the chromatoplates was eluted to give 43 mg of material which could not be separated further by tlc (recovered 37 mg), but was evidently a mixture, judging from its pasty character. An ether solution of the product was diluted with pentane to give plate clusters which were recrystallized from acetone–hexane. The product (12 mg), mp 176–180°, showed λ_{max}^{EtOH} 222 mμ (ε 10,000). After a second recrystallization the crystals (10 mg) then melted at 181–183° but their nmr spectrum indicated that they contained two major components. This fact revealed itself by two or, perhaps, three vinyl signals (4.55, 4.45, 4.40? ppm), two O-methyl signals (3.65, 6.33 ppm), two acetyl signals (2.03, 2.01 ppm), and six recognizable peaks in the C-methyl region (84, 90, 92, 97, 99, and 105.5 Hz [100-MHz spectrum]). Glpc under the conditions shown in footnote *a* of Table I showed two components (3:7) with retention times of 1.03 and 1.17, respectively, relative to cholestane. An additional recrystallization did not change this composition. Silica tlc showed that methyl cassaiate 3-acetate had a significantly greater R_f value than did this unknown mixture.

Methyl 14-Epicassaidate 3-Acetate (17) and Methyl 7,14-Di-epicassaidate 3-Acetate (20). A partial solution of 50 mg of methyl 14-epicassaiate 3-acetate (**15**) in 5 ml of 95% ethanol was treated with 50 mg of solid sodium borohydride with stirring at room temperature. All solid had dissolved within 10 min. After 2 hr, 2 *N* hydrochloric acid was added dropwise until no more gas was evolved and the resulting clear solution was concentrated to a residue under reduced pressure. The residue was partitioned between ether and water, and the ether layer was separated and concentrated. This residue was wet with a few drops of methanol, diluted with chloroform, and streaked onto an 8 × 16 in., alumina-coated chromatoplate. Development with 1:9 tetrahydrofuran–methyl dichloride gave two clearly separated, ultraviolet-absorbing bands.

The more polar band was eluted and the eluate concentrated to give a crystalline residue together with a trace of oil. The oil was washed away with three 0.1-ml portions of pentane. The resulting 30 mg (60%) of crystalline solid was rechromatographed on an 8 × 16 in. silica chromatoplate using the same solvent mixture as above to remove perhaps a trace more impurity. Recrystallization of the resultant solid from boiling ether by repeated addition of pentane gave colorless blade clusters of **methyl 8,14-diepicassaidate 3-acetate (20)** whose melting point was inadvertently not determined; $[\alpha]^{25}D$ −89°; λ_{max}^{KBr} 2.86, 2.94, 5.79 (sh), 5.83, and 6.11 μ.

Anal. Calcd for $C_{23}H_{36}O_5$: C, 70.37; H, 9.24. Found: C, 70.6; H, 9.3.

The less polar band from the plate chromatography was eluted to give 11 mg (22%) of oily **methyl 14-epicassaidate 3-acetate (17)**; $\lambda_{max}^{CS_2}$ 2.79, 2.92, 5.79, 5.84, and 6.12 μ.

2β,9β-Dihydroxy-8α-methylpodocarpan-7-one (4) from Methyl 14-Epicassaidate 3-Acetate (17). A solution of 10 mg of methyl

14-epicassaidate 2-acetate in 1 ml of acetic acid and 1 ml of ethyl acetate at 0° was treated with approximately four times the theoretical quantity of ozone. This solution was allowed to stand cold for 20 min and was then stirred for 30 min with 0.4 g of powdered zinc at room temperature. Filtration and concentration of the filtrate under reduced pressure afforded an oily residue which was purified on a silica-coated chromatoplate using pure ether for development.

The less polar of the two ultraviolet-absorbing bands from the plate was the less intense of the two bands and afforded ~0.5 mg of **2β-hydroxy-8β-methylpodocarpane-7,9-dione acetate (6)** which had an R_f value (on silica with 100% ether) and an infrared spectrum identical with those of an authentic sample.

The more polar band from the chromatoplate contained the major product from the reaction, **2β,9β-dihydroxy-8β-methylpodocarpan-7-one 2-acetate (18).** This oil, shown to be 95–97% pure by tlc, was dissolved in 2 ml of methanol and 0.5 ml of 2 *N* aqueous sodium hydroxide, and the solution was heated under reflux for 1.5 hr. It was concentrated by warming in a stream of nitrogen, and the residue was extracted three times with ether. The crude product from these extracts was purified on a silica chromatoplate developed with tetrahydrofuran–methylene dichloride (1:9). The main band from the chromatoplate was extracted, and the oily product from it was dissolved in 3 drops of tetrahydrofuran. Hexane was added in increments with boiling until the tetrahydrofuran was substantially all removed. Cooling caused precipitation of an amorphous solid which crystallized when seeded with **2β,9β-dihydroxy-8α-methylpodocarpan-7-one (4).** The perhaps 0.5 mg of crystalline product melted at 176–181° (authentic **4** melts at 180–182°), and its infrared spectrum was superimposable upon that of authentic 2β,9β-dihydroxy-8α-methylpodocarpan-7-one. The R_f value of this product on a silica chromatoplate was also identical with that of **4**.

2β,9α-Dihydroxy-8β-methylpodocarpan-7-one (21) from Methyl 7,14-Diepicassaidate 3-Acetate (20). Methyl 7,14-diepicassaidate 3-acetate (15 mg) was subjected to ozonolysis in the manner just described for methyl 14-epicassaidate 3-acetate (**17**). The less polar of the two ultraviolet-absorbing bands contained the minor product (1.5 mg) which was recrystallized from ether–pentane to give colorless plates, mp 148–150° and undepressed upon admixture with an authentic sample of **2β-hydroxy-8β-methylpodocarpane-7,9-dione acetate (6).** The infrared spectra and R_f values for the two compounds were identical.

The more polar band contained the major product, presumably **2β,9α-dihydroxy-8β-methylpodocarpan-7-one 2-acetate** (acetate of **21**), which was an oil with an R_f value greater than that of 2β,9β-dihydroxy-8α-methylpodocarpan-7-one 2-acetate (**3**). Its infrared spectrum was quite sharp and compatible with the assigned structure. This oily acetate (4.5 mg) was then hydrolyzed by heating it with 2 ml of methanol and 0.5 ml of 2 *N* aqueous sodium hydroxide for 1.5 hr. Concentration of the reaction under nitrogen followed by extraction of the product with ether gave oily **2β-9α-dihydroxy-8β-methylpodocarpan-7-one (21)** which was >98% pure at this stage of purification. The remaining impurity was removed on a chromatoplate using tetrahydrofuran–methylene dichloride (1:9) for development. The pure oil showed an infrared spectrum compatible with the assigned structure (**21**) and definitely different from that of 2β,9β-dihydroxy-8α-methylpodocarpan-7-one (**19** = **4**).

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The Preparation and Properties of *trans*-Cinnamoyl-Papain[1]

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Abstract: *trans*-Cinnamoyl-papain was prepared from the reaction of excess *trans*-cinnamoylimidazole with papain at pH 3.43 followed by gel filtration to remove the excess substrate and products. The difference spectrum of *trans*-cinnamoyl-papain *vs.* papain was determined by an indirect method since this acyl-enzyme is not stable under the conditions of its preparation. The deacylation of *trans*-cinnamoyl-papain is first order in acyl-enzyme and is identical with the rate of appearance of *trans*-cinnamic acid. The rate of the deacylation reaction is dependent on a basic group of $pK_a = 4.69$. In deuterium oxide, the rate is reduced 3.35-fold and the pK_a of the base controlling the reaction is raised 0.34 p*K* unit. Added nucleophiles including both amines and alcohols increase the rate of disappearance of the acyl-enzyme by competing with water for the acyl-enzyme. The second-order rate constants for the reactions of nucleophiles with the acyl-enzyme are larger for amines than for the corresponding alcohols; furthermore, they are dependent on the structure of the alkyl group of the nucleophile. The basicity of the nucleophile appears to be of little importance. This last observation together with the D_2O effect suggest that the group of $pK_a = 4.69$ acts as a general basic catalyst in deacylation.

In 1937 Weiss suggested that papain and other sulfhydryl enzymes catalyze certain hydrolytic and synthetic reactions *via* the intermediate formation of an acyl-thiolenzyme.[2] This suggestion, however, did not fall on fertile ground; only when the acyl-enzyme hypothesis was again suggested[3] in the early 1950's was it seriously tested and found to be valid for several proteolytic enzymes, particularly chymotrypsin.[4]

After a series of kinetic studies on papain-catalyzed reactions Smith and co-workers proposed that the acyl-enzyme mechanism applies to papain,[5] by analogy to another sulfhydryl enzyme, glyoxalase.[6] Gutfreund applied the same hypothesis to ficin-catalyzed reactions

(1) This research was supported by grants from the National Institutes of Health.

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