Rapid Acetylation of a Dihydroxy Compound by 4-(Dimethylamino)pyridine Catalysis: Application to GLC Determination of Clindamycin Palmitate Hydrochloride

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Abstract □ The acetylation of clindamycin palmitate hydrochloride, preparatory to GLC assay, was accelerated greatly by the use of 4-(dimethylamino)pyridine as a catalyst. The reaction time was reduced from 2.5 hr at 100° using pyridine and acetic anhydride to 30 min or less at ambient temperature upon addition of excess catalyst.

Keyphrases □ Clindamycin palmitate hydrochloride—GLC analysis after acetylation catalyzed by 4-(dimethylamino)pyridine □ 4-(Dimethylamino)pyridine—catalyst for acetylation of clindamycin palmitate hydrochloride preparatory to GLC assay □ GLC—analysis, clindamycin palmitate hydrochloride, after acetylation catalyzed by 4-(dimethylamino)pyridine □ Dihydroxy compounds—clindamycin palmitate hydrochloride, GLC analysis after acetylation catalyzed by 4-(dimethylamino)pyridine □ Antibacterials—clindamycin palmitate hydrochloride, GLC analysis after acetylation catalyzed by 4-(dimethylamino)pyridine

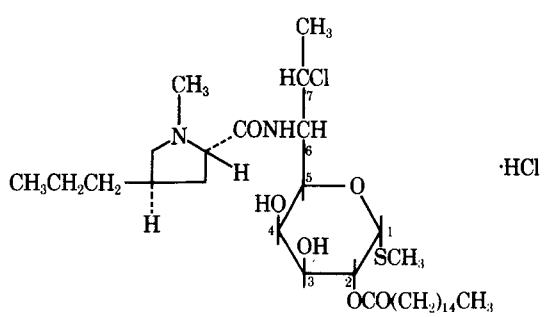
The acetylation of clindamycin palmitate hydrochloride (I) for GLC assay is catalyzed by the use of pyridine, which also acts as a solvent for the reaction (1). Pyridine forms an intermediate with acetic anhydride, acetylpyridinium ion (2), which then reacts with the hydroxyl groups at the 3- and 4-positions of clindamycin palmitate hydrochloride. The reaction requires 2.5 hr at 100° for completion.

4-(Dimethylamino)pyridine (II) recently was reported to be a much better catalyst than pyridine for various acylations (2, 3). The present study compares the rate of acetylation of I in pyridine in both the presence and absence of II.

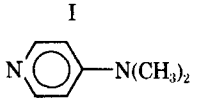
EXPERIMENTAL

Materials—All materials were analytical reagent grade.

GLC Conditions—A gas chromatograph[1] equipped with a flame-ionization detector and an electronic integrator[2] was used. The glass U-shaped column, 3 mm × 60 cm, was packed with 1% SE-54 on Gas Chrom Q (100–120 mesh). The column was conditioned for 1 week at 295° under low helium flow. The operating temperatures were: column, 275°; detector, 290°; and flash heater, off. Helium was used as the carrier gas at about 60 ml/min. One microliter of sample was injected, and the attenuation was adjusted to give peak heights averaging about 50% of the chart heights.



I



II

Internal Standard Solution—A solution of trilaurin in chloroform was used at a concentration of 5 mg/ml.

Procedure—About 75 mg of I was accurately weighed and dissolved in 5 ml of double-distilled water. One milliliter of 30% sodium carbonate solution and 5 ml of internal standard were added, and the solution was shaken vigorously for 10 min and centrifuged to form distinct layers. The aqueous layer was aspirated off, and 1-ml portions of the chloroform layer were transferred to 15-ml centrifuge tubes. One milliliter of acetic anhydride and 1 ml of pyridine were added to the tubes, and the tubes were stoppered and allowed to react at the appropriate temperature.

In the standard method, the reaction was carried out in an oven at 95° and sampled at various intervals. In the modified method, II was dissolved in pyridine at a concentration of 15 mg/ml. One milliliter of this solution (instead of pure pyridine) was used for the acylation medium. The tubes were held at ambient temperature (23–26°) and sampled for GLC injection at various times.

RESULTS AND DISCUSSION

The progress of acetylation of I using II as a catalyst at room temperature is compared to the pyridine-catalyzed reaction at 95° in Fig. 1. The GLC peak for diacetylated I becomes sharper as the reaction progresses. The disappearing peak on the tailing edge of the main peak is the 3-monoacetate ester of I. It was reported (4) that the 3-hydroxy group is the least sterically hindered hydroxyl group in the lincomycin[3] molecule

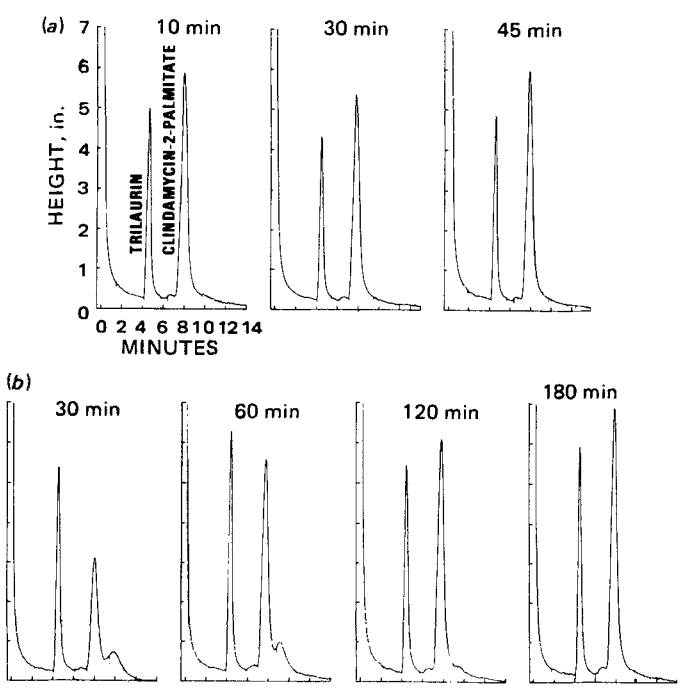
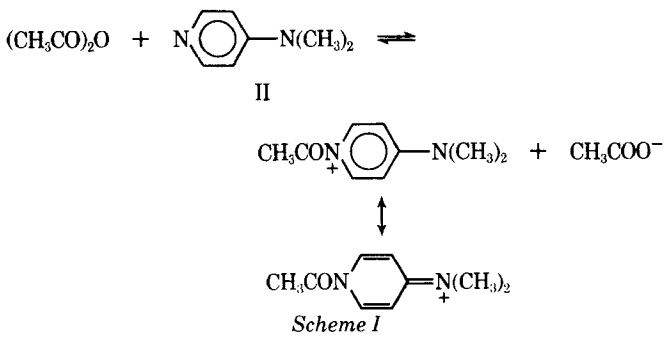


Figure 1—*Gas chromatograms illustrating the acetylation of I. Key: a, 4-(dimethylamino)pyridine added to pyridine, reaction at room temperature; and b, pyridine alone, reaction at 95°.*

[1] F & M model 402.

[2] Infotronics model CRS-104.

[3] Clindamycin is the 7-deoxy-7-(*S*)-chloro analog of lincomycin.



Scheme I

and, therefore, the most reactive. The 4-hydroxy group reacts more slowly and is the controlling factor in the diacetylation of I. The shoulder on the leading edge, always present even in highly purified lots of I, is the acetate esters of clindamycin B-2-palmitate (the 4′-ethyl analog of I) and clindamycin 3-palmitate, as indicated by Brown (1). In practice, this latter peak is not integrated with the main peak.

The acetylation reaction, using II as a catalyst, was virtually complete within 30 min at ambient temperature (Fig. 1*a*). In the absence of II, the reaction was not complete until well after 2 hr at 95° (Fig. 1*b*).

The quantitative GLC data are given in Table I. Clearly, the two procedures differed considerably in their reaction rates. Even after 26 hr, the peak area ratio for the II-catalyzed reaction was still at the plateau value without significant change.

The complete acetylation of I is a two-step reaction as already described. Acetylation of the more labile 3-hydroxy group is fast, so the reaction is controlled primarily by the second acetylation of the 4-hydroxy group. The rate of reaction is dependent on the concentrations of I, II, pyridine, and acetic anhydride. Since the latter three reagents are present in large excess compared to I, they can be considered constant. Therefore, the reaction can be approximated by the simple first-order expression:

$$-\frac{d[I]}{dt} = k_{obs}[I] \qquad \text{(Eq. 1)}$$

In integrated form, expressing concentration in percent:

$$\log \frac{100[I]}{[I_0]} = \log 100 - k_{obs}t \qquad \text{(Eq. 2)}$$

where $[I_0]$ is the initial concentration of I, and [I] is the concentration at time t.

In Fig. 2, the data are plotted according to Eq. 2. The slopes differ by a factor of about nine. In other words, even with a temperature difference of 70°, the room temperature reaction using II as a catalyst was about nine times faster than the pyridine-catalyzed reaction at 95°. If the temperatures were equal, the differences would be several orders of magnitude. Connors and Albert (2) found that the rate constant using II was 2×10^4 greater than that of the corresponding pyridine-catalyzed acetylation of 2-propanol at 54°.

The intercepts from least-squares regression analysis of the data are considerably less than 100%, as can be seen from Fig. 2. This result is due to the stepwise nature of the acetylation, which is better represented by two consecutive first-order reactions rather than by a single first-order reaction. If sufficient early time points were available, it would be apparent that there is a steep slope initially which would intersect the y-axis at about 100%.

The mechanism of the catalytic action of pyridine involves formation of an intermediate with acetic anhydride. The acetylpyridinium ion then undergoes nucleophilic attack by the hydroxy group. There is spectroscopic evidence that the acetylpyridinium ion has a very small stability constant because its presence is almost undetectable (5). Connors and Albert (2) suggested that the intermediate formed by II has enhanced stability due to the resonance forms made possible by the dimethylamino group (Scheme I).

The modified assay was applied to other 2-acyl esters of clindamycin ranging in chain length from 10 to 18 carbon atoms. The reaction rates were about equal to the palmitate, requiring no more than 30 min at ambient temperature in any case. Compound II should prove to be a versatile catalyst for acylation of other hydroxy compounds being prepared for GLC analysis.

Table I—Comparison of Two Procedures for Acetylation of I (GLC Assay)

Acetylation Procedure	Minutes	Sample	Peak Area Ratio	Average
Pyridine, 95°	30	1	1.476	1.249
		2	1.022	
	60	1	1.640	1.548
		2	1.512	
		2	1.492	
	120	1	1.894	1.917
		1	1.963	
		2	1.917	
		2	1.895	
	180	1	2.038	2.024
		2	2.009	
II in pyridine, room temperature	10	1	1.694	1.822
		2	2.002	
		2	1.771	
	30	1	2.050	2.120
		2	2.214	
		2	2.096	
	45	1	1.988	2.116
		2	2.227	
		2	2.134	
	60	1	1.971	2.065
		2	2.160	
	75	1	2.054	2.099
		1	2.092	
		2	2.152	
	90	1	2.093	2.108
		2	2.122	
	120	1	2.050	2.050
	26 hr	1	2.130	2.130

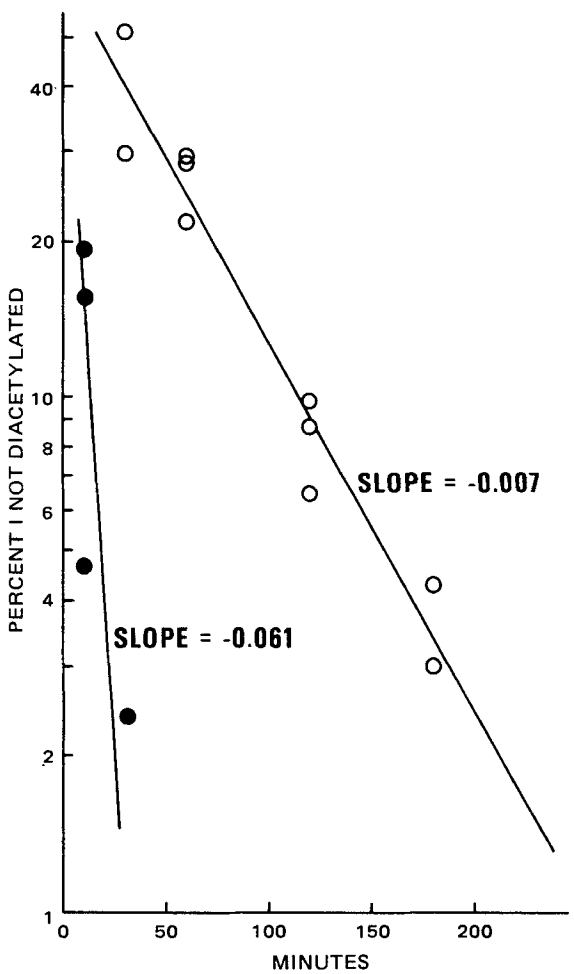


Figure 2—*Pseudo-first-order plot of acetylation of I by acetic anhydride. Key:* ○, *pyridine only, reaction at 95°; and* ●, *4-(dimethylamino)pyridine added to pyridine (15 mg/ml), reaction at room temperature.*

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Tissue Distribution of ^{3}H-Canrenoate Potassium in Rabbits

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Abstract □ Plasma and various organ concentrations of canrenone, canrenoate, and total ^{3}H-activity were measured following single doses of 20 mg of ^{3}H-canrenoate/kg iv to rabbits. Organs studied included heart, lungs, brain, kidneys, liver, adrenal glands, and spleen. Canrenoate was shown to be in rapid equilibrium with canrenone. Both were eliminated from plasma and other tissues with a half-life of about 1 hr. Plasma concentrations of both drugs were equal as early as 10 min after intravenous drug administration. Canrenone was concentrated about 10-fold in organ tissues when compared to plasma, while no such preferential uptake was found with canrenoate. Total ^{3}H-activity declined slowly in all tissues with a half-life of approximately 15 hr, indicating extensive metabolism and metabolite retention in the rabbit.

Keyphrases □ Canrenoate potassium—tissue distribution and metabolism, radiochemical study, rabbits □ Distribution, tissue—canrenoate potassium, radiochemical study, rabbits □ Metabolism—canrenoate potassium, radiochemical study, rabbits □ Radiochemistry—study of tissue distribution and metabolism, canrenoate potassium, rabbits □ Aldosterone antagonists—canrenoate potassium, tissue distribution and metabolism, radiochemical study, rabbits

The antimineralocorticoid compounds spironolactone[1] (I) and canrenoate potassium (III) are useful diuretic agents (1). Spironolactone is rapidly metabolized *via* a 7α-thiol derivative (2) to canrenone (II), a major metabolite, which exists in enzymatic equilibrium with III (Scheme I). The pharmacokinetics of I–III were investigated in rats (3), dogs (4), and humans (5, 6). Data on the kinetic disposition and further metabolism of II and III are scant (2, 7). Large differences between the tissue distribution of the lipophilic II and the water-soluble III can be expected and should be considered in the interpretation of plasma II and III levels. In this study, the tissue distribution of III potassium was investigated in rabbits.

EXPERIMENTAL

Chemicals—Pure crystalline samples of I and III were used[2], and II was prepared from III as previously described (8). ^{3}H-Canrenoate potassium[3], with a specific activity of 860 μCi/mg, was purified by solvent extraction (8) and TLC[4] in dichloromethane–methanol (8:2) prior to use. All reagents and chemicals were spectroquality.

Analytical Procedures—The fluorometric determination of II and III was performed as described previously (9). This method was specific for II and III in the presence of all other metabolites of III (8). Tritium measurements were performed by liquid scintillation counting as described previously (8). Efficiency was measured by the channels ratio method.

Rabbit Protocols—*Serial Blood Sampling*—Female New Zealand White rabbits, 2–2.5 kg, were used. Each rabbit was prepared for blood sampling by insertion of a polyethylene 50 catheter about 4 cm into an ear artery. Doses of 5, 10, and 20 mg of III/kg in aqueous solution were given by venous injections into the opposite ear. Blood samples of 2 ml each were taken with heparinized syringes at appropriate time intervals and centrifuged, and the plasma was frozen for subsequent analysis.

Tissue Analysis—Doses of 20 mg of ^{3}H-III/kg, with a specific activity of 0.2–0.5 μCi/mg, were given intravenously into an ear vein. Rabbits were sacrificed by decapitation at the following times after drug administration: 5, 10, 20, 30, 60, and 90 min and 2, 4, 8, 16, and 32 hr. One animal was used per time interval, with the exception of the 2-hr experiments where three animals were used.

Blood was collected by exsanguination from the abdominal aorta. The heart, lungs, brain, kidneys, liver, adrenal glands, and spleen were re-

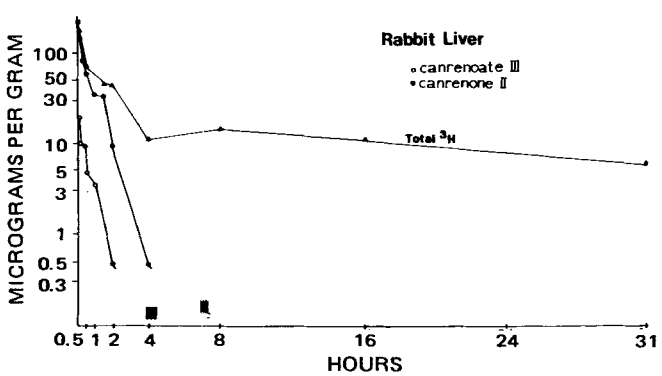

Figure 1—*Concentrations of total ^{3}H-radioactivity measured by liquid scintillation counting and concentrations of II and III measured by a specific fluorescence assay in rabbit liver.*

[1] Aldactone, Searle and Co.

[2] Boehringer Mannheim G.M.b.H.

[3] G. D. Searle and Co.

[4] Silica gel K C F254 Merck plates were used.