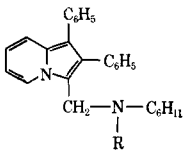
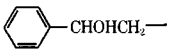


Table I—Mannich Bases

Compound	R	M.p.	Yield, %	Formula	Anal. Calcd.	Anal. Found
I		182–183°	85	$C_{33}H_{38}N_2$	C, 85.67 H, 8.28 N, 6.05	C, 85.72 H, 8.32 N, 6.18
II	CH_3CH_2—	123–124°	90	$C_{29}H_{33}N_2$	C, 85.25 H, 7.89 N, 6.86	C, 85.32 H, 7.92 N, 6.69
III	CH_3—	112–113°	88	$C_{28}H_{30}N_2$	C, 85.24 H, 7.66 N, 7.10	C, 85.36 H, 7.66 N, 7.09
IV	$(CH_3)_2CH$—	127–128°	84	$C_{30}H_{34}N_2$	C, 85.26 H, 8.11 N, 6.63	C, 85.42 H, 8.09 N, 6.42
V	$NCCH_2CH_2$—	152–153°	92	$C_{30}H_{31}N_3$	C, 83.10 H, 7.21 N, 9.69	C, 83.13 H, 7.19 N, 9.48
VI	$HOCH_2CH_2$—	138–139°	74	$C_{29}H_{32}N_2O$	C, 82.04 H, 7.60 N, 6.60	C, 82.11 H, 7.65 N, 6.78
VII		137–138°	84	$C_{35}H_{36}N_2O$	C, 83.96 H, 7.25 N, 5.59	C, 83.92 H, 7.31 N, 5.42

the crystalline product appeared. The product was collected and recrystallized from acetone. Each compound gave a negative color reaction with *p*-dimethylaminobenzaldehyde reagent, indicating that substitution had occurred at the C-3 position (5).

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COMMUNICATIONS

Time Integral of Drug Concentration in the Central (Plasma) Compartment

Keyphrases ☐ Drug concentration time integral—central compartment ☐ Absorption comparison method—different formulations

Sir:

A well-known result in pharmacokinetics is that the time integral of the concentration of a drug in the central (plasma) compartment is equal to the total amount of drug absorbed divided by the product of the volume of distribution for the compartment and the elimination rate constant. The result is of great practical importance in that amounts of drug absorbed from different formulations of a drug can be readily compared by administering the different formulations to the same subject. Standard statistical designs, such as balanced incomplete block designs, can thus be employed. The result has been proved for one-compartment and two-compartment systems under suitable conditions. The usual procedure has been to obtain an expression for the concentration in the central compartment (by solving the appropriate differential equations) and to integrate this expression over time to obtain the stated result. A recent example of this procedure is given in Eqs. 11*a* through 14*a* of Gibaldi *et al.* (1). The purpose of the present note is to show that the result is a direct consequence of two basic assumptions and thus holds under quite general conditions. In fact, the present proof is implicit in the derivation given for nonintravenous routes of administration in Eq. 22*a* of the reference.

The two basic assumptions are:

1. Elimination of the drug takes place only from the central compartment, that is, the compartment over

which the time integral of concentration can be estimated by suitable sampling techniques.

2. Elimination from this compartment is first order.

Suppose that the amount of drug in the central compartment at any time is X, that the volume of distribution for the compartment is V, and that the elimination rate constant is k_e. Then the rate of elimination is k_eX, and the amount of drug eliminated between times t and $(t + dt)$ is $k_eX\,dt$. Consequently the total amount of drug absorbed (D)—which must equal the total amount eliminated—is given by

$$D = \int_0^\infty k_eX\,dt \qquad \text{(Eq. 1)}$$

Dividing both sides of the equation by k_eV and defining the concentration C as X/V give the required result:

$$D/k_eV = \int_0^\infty C\,dt \qquad \text{(Eq. 2)}$$

As is clear from its derivation, the result is independent of the method of administration of the drug and is true for a system comprising any number of compartments with any type of transfer between them, provided only that the two basic assumptions are true. The same argument yields an analogous result for nth-order elimination from the central compartment (*i.e.*, rate of elimination $= k_eX^n$ where $n \neq 0$). In this case, it is seen that

$$D/k_eV^n = \int_0^\infty C^n\,dt \qquad \text{(Eq. 3)}$$

(1) M. Gibaldi, R. Nagashima, and G. Levy, *J. Pharm. Sci.*, **58**, 193(1969).

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After this communication was accepted for publication, it was brought to the author's attention that this method of derivation is implicit in some earlier papers; *e.g.*, J. G. Wagner *et al.*, *Nature*, **207**, 1301 (1965).

Acospectoside A III: Selective Conversion into Acovenoside B Using Snail Enzyme with Inhibited Esterase Activity

Keyphrases ☐ Acospectoside A, hydrolysis—snail enzyme ☐ Acovenoside B formation—acospectoside A hydrolysis ☐ TLC—identity ☐ Paper chromatography—identity ☐ IR spectrophotometry—identity ☐

Sir:

The use of snail (*Helix pomatia*) enzyme preparation for the hydrolysis of glucose residues in cardenolide glycosides, first advocated by Reichstein *et al.* (1), is well known. In the author's structural studies (2, 3) on acospectoside A (I) (4), the cleavage of the terminal glucose residue could not be realized using several known glucosidase preparations, *i.e.*, emulsin, strophanthobiase, β-glucosidase, invertase, taka-diastase, and cellulase. However, by using the snail enzyme the splitting of glucose could be accomplished, though to a small extent leading to acovenoside B (II) (5, 6) and the hydrolysate contained acobioside A (III) (7) and acovenoside A (IV) (5, 6) and glucose as major products. It was further shown that II and III result by cleavage of the terminal glucose residue and C-1 ester group, respectively, while IV results by subsequent hydrolysis of II and III with esterase and β-glucosidase, respectively. These and other data enabled the determination of the structure of acospectoside A as 1-*O*-acetyl-acobioside A (I).

The low yield of II may be attributed to the higher order of activity of the esterase component, as compared to the β-glucosidase component, of the snail enzyme preparation. To secure higher yields of II, it was thought that by blocking the esterase component of the mixture the β-glucosidase activity might consequently be favored. This was actually realized by "poisoning" the esterase component by employing a commercial insecticide preparation[1] containing 2,2-dichlorovinyl dimethyl phosphate (a choline esterase inhibitor) as an active ingredient. The result was the formation of acovenoside B (II) as the sole crystalline product in about 27% yield. The identity of the product was established by direct comparison (mixed melting point, TLC, paper chromatography, and IR spectra) with an authentic sample.

In natural product studies, the need is frequently encountered to effect selectively the cleavage of an ester or a glycosidic linkage in the presence of the other. The availability of an enzyme preparation, such as that of snail, which would effect both, and a method to inhibit one and thus promote the other[2] have obvious advantages in structure elucidation work. A recent example of the application of the herein reported technique is the case of gymnemic acid A, a complex triterpenoid ester glucuronoside with reported (9) antisweet and antiviral activities, in which Rao and Sinsheimer (10, 11) induced selective sugar cleavage with the snail enzyme without affecting the ester groups and thus obtained only one product. This stands in contrast to the mixture of at least four components obtained earlier by Stocklin *et al.* (12) by treatment of their gymnemic acids with snail enzyme. The utility of the method was also tested by Rao and Sinsheimer (10, 11) in three other instances, gymnemic acids B, C, and D, which again afforded single genins designated K, N, and M.

[1] Real-Kill, an insecticide marketed by Real-Kill Products, Division of Cook Chemical Co., Kansas City, Mo., was employed as esterase inhibitor. In a typical procedure, a mixture of 75.6 mg. of I, 70 mg. of snail enzyme (Helicase, marketed by Industrie Biologique Francaise, S. A. Gennevilliers, France), and 0.4 ml. of Real-Kill in 12 ml. of water was left with stirring at room temperature for 5 days.

[2] Another method has recently been reported (see *Reference 8*) for the selective inhibition of the β-glucosidase activity in the snail enzyme which resulted in enhanced esterase activity. The results are being separately published.