determined by the Lane–Eynon copper-reduction method.[7] D-Glucose pentaacetate and dihydroxyacetone diacetate included in Dianin's compound were determined by analyzing the inclusion compound for acetyl content according to the method of Green and Perkin.[8] Dianin's compound did not interfere in either of these analyses. Glycerol and sorbitol included in a host were determined by analysis of the products from periodic acid oxidation of the carbohydrate[9] after the inclusion compounds were dissolved to release the carbohydrate. The amount of the dihydroxyacetonyl acetonide included in Dianin's compound was determined by the weight increase of the host compound. The quantity of L-ascorbic acid included in Dianin's compound was estimated by comparison with known quantities of L-ascorbic acid on paper chromatograms.

Oxidation.—The inclusion compounds of D-glucose, sorbitol, and glycerol with Dianin's compound and of sorbitol and glycerol with β-cyclodextrin containing from 0.0003 to 0.01 mole of carbohydrate were suspended separately in about 10 ml. of water. Ferrous sulfate (0.0001 g. for Dianin's compound and 0.005 g. for β-cyclodextrin) and hydrogen peroxide in mole ratios of 1:1 and 1:10 (hydrogen peroxide–carbohydrate) were added to the slurry. The mixture was stirred for 1 hr. and then allowed to stand for 3 hr. at room temperature. The crystalline material was collected by filtration, washed with water, and dried. Solutions and solids were examined chromatographically using butanol–ethanol–water (40:11:19, v./v./v.) as the irrigant. Most inclusion compounds produced oxidation products identical with those obtained from oxidations of the corresponding unincluded carbohydrates under the same conditions. Only glycerol included in Dianin's compound was unaffected.

Slurries (in dilute acetic acid) of D-glucose, sorbitol, and glycerol included in Dianin's compound were treated with lead tetraacetate following the procedures of Perlin and Brice.[10] Oxidation occurred only with D-glucose and sorbitol but not with glycerol as was indicated by paper chromatographic examination of the oxidation product.

Oxidation of carbohydrates included in Dianin's compound and in β-cyclodextrin with periodic acid were carried out according to a procedure of Miner and Dalton.[9] The formic acid produced was determined quantitatively and considered as an indication of the extent of oxidation.

(7) (a) J. Lane and L. Eynon, *Soc. Chem. Ind.* (London), **42**, 32T, 143T, 463T (1923); **44**, 150T (1925); **46**, 434T (1927); **50**, 85T (1931); (b) "Polarimetry, Saccharimetry, and the Sugars," Circular C-440, United States Government Printing Office, Washington, D. C., 1942, p. 185.

(8) A. G. Green and A. G. Perkin, *J. Chem. Soc.*, **89**, 811 (1906).

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Studies on the Alkaloids of *Securinega virosa* Pax. *et* Hoffm. IV.[1] The Preferred Conformations of Allosecurinine (Phyllochrysine) and Dihydrosecurinine

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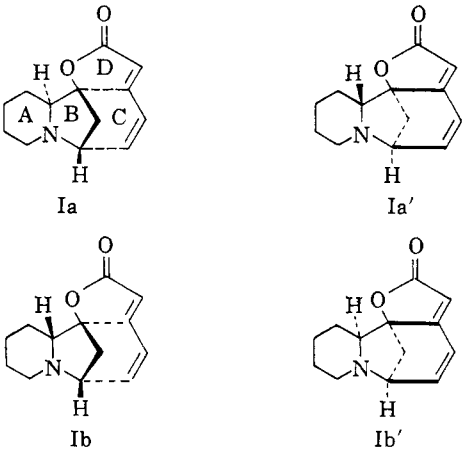
In the preceding publications,[1] we proposed the absolute configuration Ia for virosecurinine, Ib′ for allosecurinine (phyllochrysine),[2] and Ia′ for securinine. This assignment is not in agreement with that suggested recently by Parello, *et al.*,[2] on the basis of the relative rates of reaction of these alkaloids with methyl iodide, but the nuclear magnetic resonance evidence is more compatible with our proposed structure, as was already pointed out by us in a recent paper.[3] Our conclusion was later supported by Horii, *et al.*,[4] who also proposed the absolute configuration Ia′ for securinine, an antipode of virosecurinine.



Parello, *et al.*,[2] examined the ultraviolet absorption spectra of allosecurinine and securinine and suggested that while securinine possesses the conformation b-1 (or a mirror image of b-1), allosecurinine exists in the conformation a-2 (or a mirror image of a-2). The same ultraviolet spectral observation was also made by Horii, *et al.*,[4] who reported that the conformation of a mirror image of a-1[5] is preferred for securinine. Both these authors noted that the characteristic absorption at 330 mμ in the ultraviolet spectrum as well as the yellow coloring of securinine would originate most probably in transannular interaction from the nitrogen to the conjugated system in this alkaloid. However, in the conformation of a-2 (or a mirror image of a-2) assigned by Parello, *et al.*, for allosecurinine, no interaction can arise between the electron pair of the nitrogen atom and the conjugated system because of an intervening methylene bridge, even if the ring A adopts a boat form.[2] In view of this contradictory conclusion made by Parello, *et al.*, we have examined in some detail the ultraviolet absorption spectrum of allosecurinine, in comparison with that of virosecurinine, and the effects of solvents on their absorption spectra were studied. Allosecurinine and virosecurinine exhibited in ethanol solution two absorption maxima at 256 mμ (log ε 4.04),[6] 306 (3.11), 254.5 (4.21),[6] and 331 (3.26), respectively. The wave length and the maximum extinction coefficient of the lower wave length absorption bands in both these alkaloids are affected by a change of

(1) The paper which was published by T. Nakano, T. H. Yang, S. Terao and L. J. Durham [*Chem. Ind.* (London), 1034 (1963)] represents part III of this series; for part II see T. Nakano, T. H. Yang, and S. Terao, *J. Org. Chem.*, **28**, 2619 (1963).

(2) J. Parello, A. Melera, and R. Goutarel, *Bull. soc. chim. France*, 898 (1963). According to a private communication from Dr. Parello, phyllochrysine was shown by direct comparison to be identical with allosecurinine.

(3) T. Nakano, T. H. Yang, S. Terao, and L. J. Durham, *Chem. Ind.* (London), 1763 (1963).

(4) Z. Horii, M. Ikeda, Y. Yamawaki, Y. Tamura, S. Saito, and K. Kodera, *Tetrahedron*, **19**, 2101 (1963).

(5) In this case, according to whether the electron pair of the nitrogen atom is either equatorial or axial in respect to ring A, two conformers are possible.

(6) As was already reported, this absorption band is due to the α,β,γ,δ-unsaturated five-membered lactone moiety [see T. Nakano, T. H. Yang, and S. Terao, *Tetrahedron*, **19**, 609 (1963)].

solvents, and as the polarity[7] of the solvent increases these bands are shifted to the lower wave length region, and upon addition of acid they disappear. It should be noted here that in carbon tetrachloride solution allosecurinine absorbs at a higher wave length[8] (342 mμ) than virosecurinine (332 mμ), but when the spectrum was measured in ethanol solution the absorption maximum of allosecurinine undergoes a significant shift to 306 mμ, due to the hydrogen-bond formation with ethanol, whereas that of virosecurinine does not move appreciably to a lower wave length under the same condition (see Table I).

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA[a] OF ALLOSECURININE AND VIROSECURININE

Solvent	Allosecurinine λ_{max}, mμ	Allosecurinine log ε	Virosecurinine λ_{max}, mμ	Virosecurinine log ε
CCl_4[b]	342	3.17	332	3.30
99.5% EtOH	256	4.04	254.5	4.21
	306	3.11	330–332	3.26
95% EtOH	256.5	4.19	255	4.22
	304[c]	3.36	325	3.35
50% EtOH	259	4.20	257.5	4.23
	299[c]	3.37	300[c]	3.44
H_2O	259	4.24	257.5	4.25
	299[c]	3.39	300[c]	3.46
$H_2O + H^+$	256.5	4.26	256.5	4.26

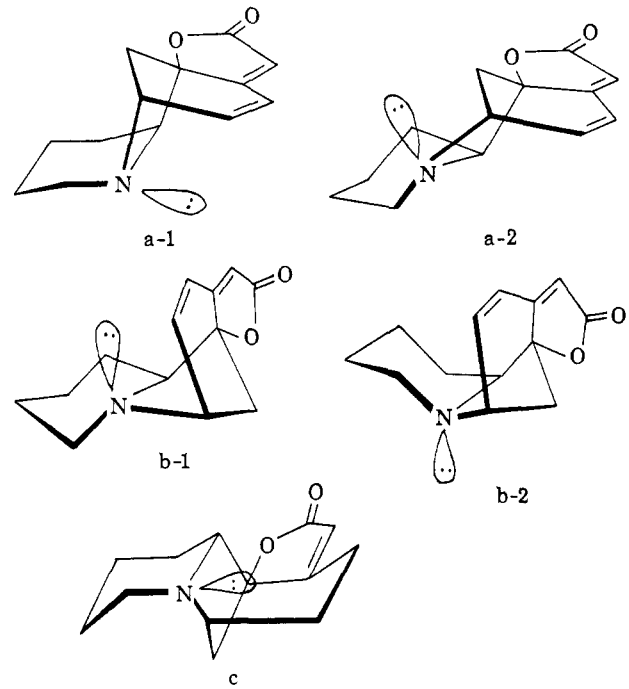
[a] The spectra were measured with a Shimazu self-recording ultraviolet spectrophotometer "SV-50." [b] In this case, the first absorption maximum could not be measured since carbon tetrachloride itself is not transparent below 300 mμ. [c] Shoulder.

In general, absorption due to an n → π* transition[9] is weak and the molar extinction coefficient (log ε) of its maximum absorption is of the order of magnitude of 1 to 2. It should be pointed out, therefore, that the abnormal enhancement[10] (log ε 3.11–3.35) of the n → π* transitions in both allosecurinine and virosecurinine must be attributed to the overlapping of the nonbonding p-orbital of the nitrogen atom with the π-orbitals of the ground state of the α,β,γ,δ-unsaturated five-membered lactone system by homoallylic conjugation.

The circular dichroism as well as the optical rotatory dispersion measurement also confirmed the above observation. While virosecurinine methiodide shows only a plain rotatory dispersion curve, both virosecurinine and allosecurinine exhibit strong Cotton effects, which upon addition of acid disappear. That the origin of these Cotton effects[11] may be ascribable to the n → π* transitions in these systems was clearly detected by the respective circular dichroism curves. As is apparent from the Dreiding models, the conformation of either a-2 or b-2 does not satisfy the steric requirements for this sort of interaction, and we favor the conformation b-1 for allosecurinine and a-1 for virosecurinine.

Parello, *et al.*,[2] observed from a study of the rates of methiodide formation for securinine and allosecurinine that securinine reacts with methyl iodide 27 times slower than allosecurinine, the rate constants being 1.77×10^{-4} and 46.0×10^{-4} sec.$^{-1}$, respectively. This difference in the reaction rate between these alkaloids suggests that in the case of securinine (and virosecurinine) in which the A/B ring junction is *cis*, the electron pair of the nitrogen atom is somewhat more sterically hindered than that in the case of allosecurinine whose A/B ring junction is *trans*. This steric situation may account for the fact that while in allosecurinine the second ultraviolet absorption maximum undergoes a blue shift of 36 mμ due to hydrogen bonding, on change from a nonpolar (carbon tetrachloride) to a hydroxylic solvent (ethanol), in virosecurinine (and securinine) it does not move appreciably to a lower wave length under the same condition (see Table I).

Our attention was next turned to dihydrosecurinine, which we isolated as a minor alkaloid from *Securinega suffruticosa* Rehd.[12] in order to see what conformation is favorable for it. Careful examination of the ultraviolet spectrum of dihydrosecurinine disclosed that besides an absorption maximum at 215 mμ, which is due to the α,β-unsaturated γ-lactone moiety, a somewhat weaker absorption band appears at 300 mμ (log ε 2.12), which vanishes upon addition of acid. The circular



dichroism as well as rotatory dispersion measurement also confirmed that this band results from the n → π* transition of the lone-pair electrons of the nitrogen atom to the α,β-unsaturated γ-lactone system of ring D. The steric requirements for this interaction necessitate the conformation c[13] for dihydrosecurinine.

(7) In general, absorption involving lone-pair electrons moves to higher frequencies on change from a nonpolar to a hydroxylic solvent, owing to the stabilization of the lone-pair electrons in the ground state by hydrogen bonding. The disappearance of these bands in acid solution, due to formation of cation, is an indication of a transition involving lone-pair electrons (see M. Kasha, *Discussions Faraday Soc.*, **9**, 15 (1950)).

(8) It is pertinent to mention that both allosecurinine and virosecurinine form yellow crystals, but the color of the former is much more intense.

(9) S. F. Mason, "Physical Methods in Heterocyclic Chemistry," Vol. II, A. R. Katritzky, Ed., Academic Press, London, 1963, p. 7.

(10) For similar references, see R. C. Cookson and N. S. Wariyar, *J. Chem. Soc.*, 2302 (1956); H. Labhart and G. Wagniére, *Helv. Chim. Acta*, **42**, 2219 (1959).

(11) Horii, *et al.*,[4] suggested that the Cotton effect in the optical rotatory dispersion curve of securinine may be caused by the interaction between the nitrogen and the conjugated system, but this was not confirmed by the circular dichroism measurement.

(12) T. Nakano and S. Terao, unpublished work. The absolute configuration of this alkaloid is already known since it is antipodal with dihydrovirosecurinine[6]; also see ref. 4.

(13) In this case, both of the two conformers in which the electron pair of the nitrogen atom is either equatorial or axial with respect to ring A satisfies this steric requirement.

Experimental

The circular dichroism (C.D.) measurements were conducted using a Shimazu C.D. spectrophotometer "Dichrograph." The optical rotatory dispersion curves were determined by means of a Rudolph automatically recording spectropolarimeter. We are indebted to Dr. K. Kuriyama of the research laboratory, Shionogi and Co., Ltd., for the optical rotatory dispersion measurements.

Virosecurinine.—O.R.D. (*c* 0.02, methanol): $[\phi]_{589}$ +2360°, $[\phi]_{375}$ +20,200° (peak), $[\phi]_{265}$ −39,000°; C.D. (*c* 0.01, methanol): $[\theta]_{360}$[14] 0, $[\theta]_{320}$ +39,000, $[\theta]_{270}$ 0; for ultraviolet data, see Table I.

Allosecurinine.—O.R.D. (*c* 0.29, methanol): $[\phi]_{589}$ −2200°, $[\phi]_{345}$ −20,500° (trough), $[\phi]_{272}$ +58,400°; C.D. (*c* 0.01, methanol) $[\theta]_{350}$ 0, $[\theta]_{305}$ −46,000, $[\theta]_{270}$ 0; for ultraviolet data, see Table I.

Virosecurinine Methiodide.—O.R.D. (*c* 0.10, methanol): $[\phi]_{589}$ +4850°, $[\phi]_{400}$ +7200°, $[\phi]_{300}$ +53,800°, $[\phi]_{280}$ +251,000°.

Dihydrosecurinine.—O.R.D. (*c* 0.11, methanol): $[\phi]_{589}$ +19°, $[\phi]_{325}$ +528° (peak), $[\phi]_{273}$ −2170°; C.D. (*c* 0.15, methanol): $[\theta]_{330}$ 0, $[\theta]_{300}$ −2400, $[\theta]_{270}$ 0; λ_{max}^{MeOH} 215 mμ (log ε 4.21) and 300 mμ (log ε 2.12).

Acknowledgment.—This work was supported by Grant No. GM 09362-02 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(14) For nomenclature see C. Djerassi and E. Bunnenberg, *Proc. Chem. Soc.*, 299 (1963).

The Tritiation of Diethyl Benzylacetamidomalonate by Exposure to Tritium Gas

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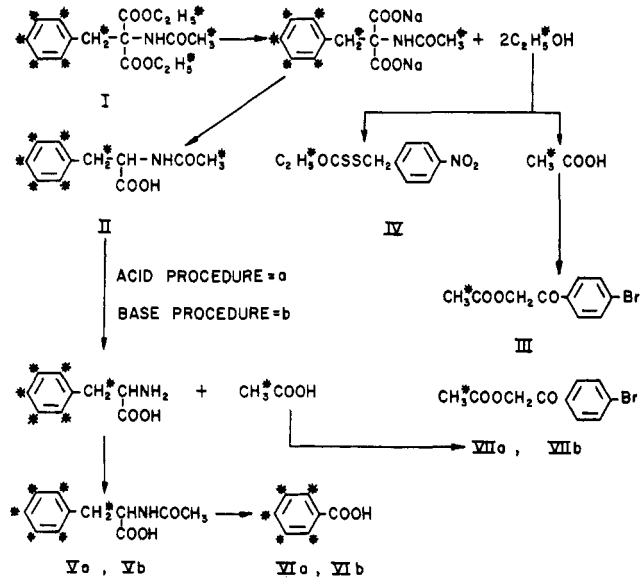
A need for tritiated *dl*-phenylalanine prompted us to apply the tritium gas exposure technique[3] to diethyl benzylacetamidomalonate, from which the desired product is obtainable by suitable degradations. With this more readily purifiable starting material and with a measure of purification inherent in the chemical degradations, it was hoped to ameliorate the difficulties that arise from trace impurities of high specific activity.[3,4] It also provided an opportunity to supplement available information[5−12] on the distribution of tritium among different classes of hydrogen atoms in tritium-irradiated solids.

SCHEME I



Two samples of tritium-irradiated diethyl benzylacetamidomalonate were purified by methods including solution in methanol, treatment with water vapor, and washing with sodium hydroxide to remove labile hydrogen; recrystallization; and repeated chromatography on activated alumina.

Each purified sample (I) was subjected to the degradation and derivatization, outlined in Scheme I, which was designed to determine activity associated with hydrogen at various positions in the molecule. Asterisks denote hydrogen atoms derived from the starting material. The degradation was straightforward, except perhaps for the reacetylation step yielding Va and Vb. This was included because in preliminary experiments with unlabeled phenylalanine, no benzoic acid could be obtained by permanganate oxidation. A good yield was obtained, however, from acetylphenylalanine. The recovery of ethanol from an aqueous solution as ethyl *p*-nitrobenzylxanthate is a little known but highly recommended procedure.

Following conversion of I to II, acidic a, as well as basic b, conditions were used to remove the acetyl group from N-acetylphenylalanine II, sample 2. This modification was incorporated after it was found that there was a loss of activity by exchange in the acetyl group under conditions of strong alkaline hydrolysis. This was not totally unexpected[13,14] and is seen in the lower specific activity of VIIb *vs.* VIIa in Table I. On the other hand, loss by exchange in the aromatic ring was expected[15] during the conditions of strong acid hydrolysis used to remove the acetyl group. Thus benzoic acid VIa showed much lower activity than VIb. Total recovery of activity was therefore calculated by using the values from both routes of degradation.

The best summation of per cent molar specific activity recovered is obtained from IV, Vb, and VIIa. This summation could not be made on sample 1 because a comparable acid hydrolysis was not done. For sam-

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(2) Operated by the University of Chicago for the United States Atomic Energy Commission. The present work was supported in part by Air Force Contract AF 33(616)-3875, Project No. 7021, Task No. 70347.

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