washed with dilute sulfuric acid, 10% sodium bicarbonate solution, water, dried and evaporated. The resulting gum (2.76 g.) was chromatographed on 85 g. of alumina[29] and led to two crystalline products.

Elution with benzene produced 350 mg. of solid (m.p. 127–137°) which was recrystallized successively from methanol, benzene–ether and ethanol to give colorless plates of the **13-nor-3-ketone XXVI**, m.p. 146–148°, $[\alpha]D$ +74° (*c* 0.43); λ_{max}^{EtOH} 224 mμ, log ε 4.11; $\lambda_{max}^{CHCl_3}$ 5.65 (lactone), 5.82 (ketone) and 5.90 μ (double bond[2]).

Anal. Calcd. for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.71, 71.87; H, 7.74, 7.76.

The 2,4-dinitrophenylhydrazone of the nor-ketone XXVI, prepared by the acetic acid method,[31] was purified by chromatography on alumina and eluted with benzene–chloroform (1:1). Recrystallization from acetic acid and from ethanol gave fine yellow needles, m.p. 188–191°, $[\alpha]D$ +178° (*c* 0.80); $\lambda_{max}^{CHCl_3}$ 364 mμ,[20] log ε 4.34; $\lambda_{max}^{CHCl_3}$ 3.00 (NH), 5.67 (lactone) and 5.90 μ (double bond[2]).

Anal. Calcd. for $C_{20}H_{22}N_4O_6$: C, 57.96; H, 5.35; N, 13.52. Found: C, 57.93; H, 5.34; N, 13.48.

The later benzene and benzene–chloroform (9:1) eluates from the original chromatogram furnished 725 mg. of crystals (m.p. 150–160°) which were subjected to recrystallization from acetone–ether, methanol and finally acetone. The resulting small prisms of the **hydroxy-aldehyde XVIIa** exhibited m.p. 164–166°, $[\alpha]D$ +39° (*c* 0.79); λ_{max}^{EtOH} 224 mμ, log ε 3.90; $\lambda_{max}^{CHCl_3}$ 2.82, 5.65, 5.82 and 5.90 μ, did not give a Tollens reaction at room temperature and did not form a trityl ether under conditions where iresin readily forms a monotrityl ether (XXVc).[22] No formaldehyde was produced under the basic conditions employed successfully with icterogenin,[21] thus excluding structure XXVIIIa.

Anal. Calcd. for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.74; H, 7.73.

A small sample (21 mg.) of the hydroxy-aldehyde XXVIIa was treated with 4 mg. of sodium borohydride[7] in ethanol solution and was then processed as described above in the reduction of iresin. Recrystallization from acetone–ether led to 16 mg. of dihydroiresin (XXIX),[2] m.p. 142–146°, diacetate,[2] m.p. 208–210°.

The presence of the alcoholic function in the hydroxy-aldehyde XXVIIa was demonstrated by the formation of the **benzoate XXVIIb**, which was recrystallized from methanol and from acetone–ether, m.p. 193–195°, $[\alpha]^{16}D$ −45° (*c* 0.96).

Anal. Calcd. for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.95; H, 6.53.

The hydroxy aldehyde was converted into the **2,4-dinitrophenylhydrazone** by the acetic acid procedure[31] and crystallized from ethanol as orange-yellow prisms, m.p. 274–278° dec., $[\alpha]D$ +19° (*c* 0.95), $\lambda_{max}^{CHCl_3}$ 354 mμ,[20] log ε 4.12, $\lambda_{max}^{CHCl_3}$ 3.00, 5.67 and 5.90 μ.

Anal. Calcd. for $C_{21}H_{24}N_4O_7$: C, 56.74; H, 5.44; N, 12.61. Found: C, 56.76; H, 5.59; N, 12.86.

DETROIT, MICHIGAN

(31) C. Djerassi, THIS JOURNAL, **71**, 1003 (1949).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF S. B. PENICK AND CO. AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Constitution of Samidin, Dihydrosamidin and Visnadin[1]

BY ERIC SMITH, NORMAN HOSANSKY, W. G. BYWATER AND EUGENE E. VAN TAMELEN

RECEIVED OCTOBER 22, 1956

Analytical and degradative evidence have been obtained which indicate the structure XIII for samidin (R = —CH=C-$(CH_3)_2$), dihydrosamidin (R = —$CH_2CH(CH_3)_2$) and visnadin (R = $CH(CH_3)CH_2CH_3$), potent vasodilatory agents isolated from the "visnagan" fraction of *Ammi visnaga*.

Ammi visnaga L. (bishop's weed), a plant indigenous to the Mediterranean regions, has been used in Egypt for centuries as a home remedy and spasmolytic. That the seeds contain biologically active substances other than the chromones khellin and visnagin[2] has been shown by Samaan,[3] whose "visnagan" fraction (the oil remaining after removal of all crystalline material) evidenced considerable vasodilatory activity. Visnagan was subsequently investigated by Cavallito and Rockwell,[4] who obtained, through chromatography on silica, a glassy solid to which the formula $C_{22}H_{26}O_7$ was ascribed. In repeating the chromatographic procedure, Smith, Pucci and Bywater[5] were able to secure two crystalline substances, designated as RI-778 (visamminol)[6] and RI-832. More recently,[5b] refined chromatographic techniques allowed isolation from the visnagan fraction of six crystalline components, of which RI-860, the so-called "yellow body," and visamminol did not possess biological activity in the test employed. The three remaining substances, all colorless and optically active, were strongly vasodilatory and consequently chemical investigations were initiated.

Elemental analyses indicated the molecular formula $C_{21}H_{22}O_7$ for substance RI-870 (m.p. 134–5°), which has been given the name *samidin*. RI-832 (m.p. 85–8°), called *visnadin*, and dihydro-RI-870 (m.p. 111–13°) are isomers possessing the formula $C_{21}H_{24}O_7$. The relationship between samidin and dihydro-RI-870 is more than empirical: the former substance absorbed one mole of hydrogen over platinum, giving rise to the latter. Therefore, dihydro-RI-870 may be properly designated *dihydrosamidin*.

The ultraviolet absorption spectra of all three substances (Table I) coincide at the maximum 323–324 mμ and at the minimum 264 mμ and are otherwise similar except that samidin absorbs strongly at lower wave lengths.

Comparison of ultraviolet curves with those of authentic chromones and coumarins provided at the outset strong evidence for the presence of a 7-oxygenated coumarin chromophore, an assignment

(1) Described preliminarily in *Chemistry & Industry*, 718 (1956).

(2) E. Spaeth and W. Gruber, *Ber.*, **71B**, 106 (1938); **74**, 1549 (1941).

(3) K. Samaan, *Quart. J. Pharm. Pharmacol.*, **4**, 14 (1931); **6**, 12 (1933); **18**, 83 (1945).

(4) C. G. Cavallito and H. E. Rockwell, *J. Org. Chem.*, **15**, 820 (1950).

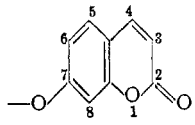
(5) (a) E. Smith, L. A. Pucci and W. G. Bywater, *Science*, **115**, 520 (1952); (b) E. Smith, N. Hosansky and W. G. Bywater, Abstracts of the Medicinal Division, 24N, 126th Meeting of the American Chemical Society, New York, N. Y., September, 1954.

(6) W. Benzce and H. Schmid, *Experientia*, **10**, 12 (1954).

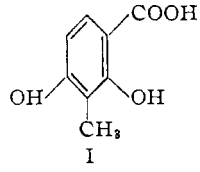
TABLE I

Substance	λmax, mμ	log ε	λmin, mμ	log ε
Samidin	324	4.17	264	3.27
Dihydrosamidin	323	4.12	264	3.16
Visnadin	323	4.14	264	3.29
7-Methoxycoumarin	323	4.12	260	3.08
Dihydroxanthotoxin	330	4.01	274	3.45

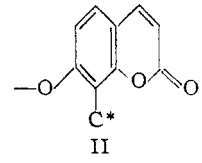
supported by the alkaline fusion results described below.[7]



Maintenance of a samidin-alkali melt at 230° for 30 minutes led to the formation of a substance $C_8H_8O_4$, m.p. 213–214° dec., which, yielding 2-methylresorcinol on thermal decarboxylation, subsequently was identified by infrared spectral comparison as 3-methyl-β-resorcylic acid (I).[8] Since samidin itself is not an acid, the carboxyl group present in the methylresorcylic acid must be masked, and, by incorporating the lactone moiety known to be present, the only logical[9] skeleton which emerges is II, where the starred carbon represents the actual or potential methyl group of the basic fusion product.



I



II

In order to continue the structural argument, it is necessary to describe certain hydrolysis operations and the study of the resulting products. In titration experiments, two equivalents of alkali were consumed by each of the coumarins at room temperature, whereas at reflux, three equivalents were used up. Apparently one equivalent is required for opening of the lactone ring; the remaining two serve to liberate volatile carboxylic acids, all of which were isolated and identified At the higher temperature, samidin surrenders senecioic acid, whereas *l*-methylethylacetic acid and isovaleric acid are obtained from visnadin and dihydrosamidin, respectively. In addition, one mole of acetic acid accompanies, in each case, the five-carbon acid. These findings, it may be mentioned in passing, define the structural difference between samidin and dihydrosamidin.

It seemed most likely, in light of the above results, that the trio of new natural products were diesters, and isolation of a glycol with the anticipated composition $C_{14}H_{14}O_5$ was indicated as a logical move. When a solution of samidin or visnadin in *ethanolic* potassium hydroxide was refluxed, an optically active substance III formed which gave, however, an analysis corresponding to the formula $C_{16}H_{18}O_5$. This same product resulted from the action of ethanolic hydrochloric acid on the parent material. Moreover, when the basic hydrolysis was carried out in *methanolic* base, a different product IV, having the composition $C_{15}H_{16}O_5$, was obtained; *and it became clear that solvent had been incorporated into the system during the saponification step.* Neither samidin nor visnadin shows the presence of alkoxyl in the Zeisel determination, and the fact that III and IV possess one ethoxyl and one methoxyl, respectively, confirms this interpretation.

The desired C_{14}-diol (V) was obtained in two ways. Either aqueous alkaline hydrolysis of samidin or ether cleavage of III with concentrated hydriodic acid afforded the crystalline, optically active product, the glycol nature of which was confirmed by the preparation of a diacetate. The hydriodic acid step is very facile in that it is completed in a matter of minutes at room temperature, and the preparation of the diol by this means is preferable to the direct, aqueous alkaline hydrolysis.

The ultraviolet spectra of all these transformation products were similar to those of the parent coumarins, indicating that the chromophores had been unmodified in any major respect during the hydrolysis steps.

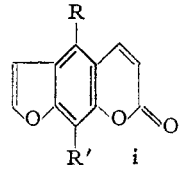
Sufficient evidence for a unique structure assignment was provided by a more detailed chemical investigation of the ethanolysis product III, which appears to be identical with the "ethylkhellactone" obtained by Spaeth, *et al.*,[10a] through alkaline hydrolysis of crude *Ammi visnaga* extracts. The analogous "methylkhellactone" and "khellactone" (diol V) were studied more recently by Schmid, *et al.*[10b] Although the latter group of investigators did not advance definite structures for the naturally occurring parent coumarins, they did establish the same structures independently deduced by us for the products III, IV, V and XI. Presented below are our experimental findings and an abbreviated version of the arguments which led to the structural assignments.

First of all, none of the coumarins secured in the entirety of this investigation gave a coloration with ferric chloride and consequently none is phenolic. Substance III was expected to be the monoether of a glycol, and this presumption was confirmed by the preparation of a monoacetate. Since the fifth oxygen in III did not reveal itself by any detectable reactivity and since the parent coumarins bear no small alkyl group attached to oxygen, the members of this group must be *cyclic ethers*, a conclusion which, in view of the apparent absence of an excess of double bonds over those in the coumarin ring

(7) The observation that each of the three substances dissolves on warming in alcoholic alkaline solution with the formation of a deep yellow color is in keeping with the coumarin formulation (E. Spaeth and H. Schmid, *Ber.*, **73B**, 1309 (1940)).

(8) The authors are indebted to Dr. J. W. Hinman, The Upjohn Co., for supplying the 3-methyl-β-resorcylic acid and 2-methylresorcinol.

(9) Xanthotoxin (i, R = H, R′ = OCH_3), on basic fusion, affords pyrogallol-4-carboxylic acid (H. Thoms, *Ber.*, **44**, 332 (1911)); bergapten (i, R = OCH_3; R′ = H) is reported to yield phloroglucinol (C. Pomeranz, *Monatsh.*, **12**, 379 (1891)).



i

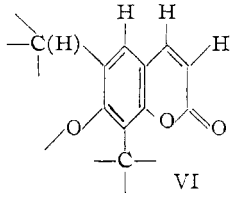
(10) (a) E. Spaeth, W. Gruber and O. Matzke, *Canadian J. Chem.*, **31**, 715 (1953); (b) W. Benzce, O. Halpern and H. Schmid, *Experientia*, **12**, 137 (1956); (c) a similar finding was reported by Schmid, *et al.* (ref. 10b).

(*vide infra*), agrees with the demands of the molecular formulas. Finally, none of these substances gives indication of aldehydic or ketonic properties, hence they are probably not hemiacetals or hemiketals.

The nature of three additional carbon atoms became apparent when it was observed that alkaline permanganate oxidation of ether III yielded acetone—a $(CH_3)_2C-O-$ grouping is necessarily present.[10c]

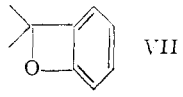
Hydrogenation of III by means of palladium-on-charcoal in glacial acetic acid led to the *dihydro product*, the ultraviolet spectrum of which indicated saturation of the 3,4-double bond of the coumarin ring. The new substance was oxidized with fuming nitric acid; succinic acid was identified as one of the products, a finding which indicates lack of substitution at the 3- and 4-positions of the parent ring system.[11]

By utilizing these results we see that partial structure II may now be expanded to VI, which is potentially tricyclic.[12]



VI

Now, any four-membered ether of the type VII is untenable because, being a benzyl ether, ring cleav-

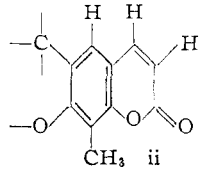


VII

age by hydrogen iodide during the conversion of III to V would be expected; on this basis the diol should be phenolic, but it is not. Also, 3-hydroxy-2,3-dihydrobenzofurans may be excluded from consideration, since they have been reported[13] to dehydrate spontaneously and give the benzofuran. Finally, by taking into account the observation that the diol consumes in an acidic medium just one mole of periodate[10c] without liberation of volatile aldehydes, we find that only one formulation, namely, IX, serves suitably as the structure of the diol.

(11) The spectral character of the natural coumarins offered no evidence for oxygenation at either of these positions, and it is apparent that hydroxyl attachment is excluded. Because of the number of oxygens which must therefore be placed on the few carbon atoms remaining after allowance is made for those in the coumarin ring and those in the $(CH_3)_2C-O-$ portion, the two methylene groups of the succinic acid *cannot* arise from any portion of the molecule other than the 3- and 4-positions of dihydro-III.

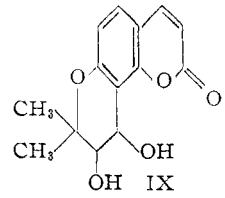
(12) Partial structure ii, which is the one following most naturally



ii

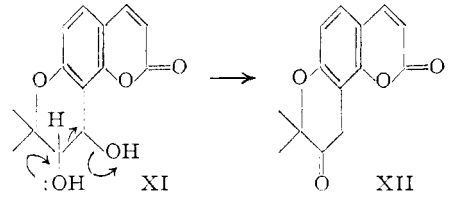
from the basic fusion results, can be dismissed at this stage in that it is not possible to develop from it a structure for diol V which has no more than one hydroxyl or ethereal oxygen per carbon atom.

(13) R. Stoermer and W. Koenig, *Ber.*, **39**, 499 (1906).



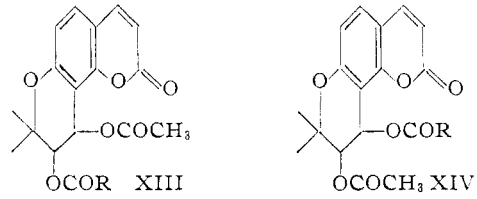
IX

Spaeth, Gruber and Matzke[10a] reported that the monoethyl ether III yielded, through the action of phosphorus pentoxide or hydrogen bromide in acetic acid, a substance (X), $C_{14}H_{12}O_4$, which was optically inactive, consumed two moles of hydrogen under catalytic conditions and formed a *p*-nitrophenylhydrazone. We obtained what appears to be the same material[10c] by dehydrating the diol V in hydrobromic–acetic acid and noted in addition that compound X gives no aldehyde or phenol color tests and displays an ultraviolet absorption which is very similar to that of its precursor. These observations provide support for the proposal IX, in that XII, a completely satisfactory expression for ketone X, arises logically from the diol structure by the process (XI → XII).[14]



XI XII

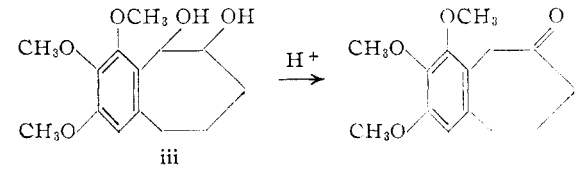
Samidin, dihydrosamidin and visnadin are mixed diesters of the diol V. In order to distinguish the two possible formulations XIII and XIV, we con-



XIII XIV

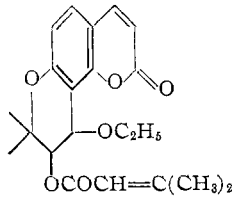
sider now the selective solvolysis of a representative, samidin. When subjected to the action of excess potassium hydroxide in ethanol *at room temperature*, this substance was converted to a new product (XV), $C_{21}H_{24}O_6$, which must have arisen through loss of acetic acid and incorporation of solvent into the molecule. The general nature of the change was confirmed by Zeisel determination (one $-OC_2H_5$), the apparent absence of alcoholic hydroxyl in XV and the liberation of senecioic acid on refluxing XV with alcoholic alkali. The incorporation of ethoxyl during hydrolysis becomes clear with the development of the diester structures pictured above. It is well recognized that certain *benzyl esters* suffer O-alkyl cleavage in either basic or

(14) Glycol iii rearranges to the β-benzsuberone (G. N. Walker, THIS JOURNAL, **77**, 6099 (1955))

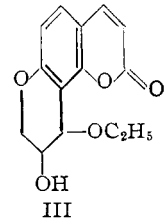


iii

acidic alcohol solution. Although not observed with esters of benzyl alcohol itself, such cleavage is facilitated by alkyl substitution on the benzyl carbon, attachment of the benzyl oxygen to a ring (*e.g.*, α-tetralol) and the presence of *o*- or *p*-electron-donating groups on the aromatic ring.[15] All three of these features are present in the structural type under consideration. *If* the loss of acetoxyl involves *simple* replacement by ethoxyl, structure XIII (R = $CH{=}C(CH_3)_2$) must represent samidin, and dihydrosamidin and visnadin may be assigned the structure XIII (R = $CH(CH_3)C_2H_5$), respectively. The partial solvolysis product XV of samidin and the monoether III may then be represented by the formulas

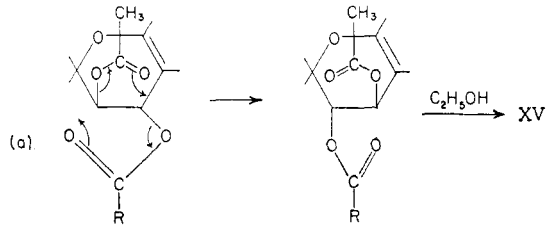


XV



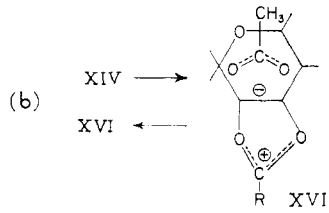
III

However, before considering the structural question settled, we must examine the possibility that the monoester XV might have come, not from XIII, but—by way of over-all acyl migration—from the alternate structure XIV (R = $CH{=}C(CH_3)_2$) for the parent natural product. There are several distinct mechanisms by which such a change might be realized. In the first (a), a concerted, twofold migration is envisioned which leads to inversion at



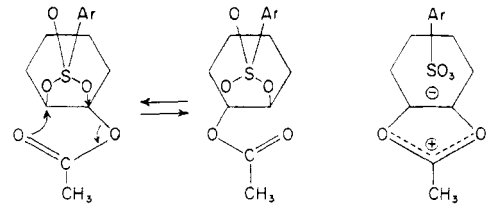
XIV XVI

both asymmetric centers and interchange of the acetyl and the senecioyl groups. Mechanism b, in which starting diester XIV is transformed to the ion pair XVII and then collapses to the isomeric diester, is similar to (a) and may be indistinguishable from it. In connection with the possible contributions of *either* mechanism a or b, it is pertinent that a closely similar case has been investigated in some detail. Winstein and Heck,[16] studying *d*-



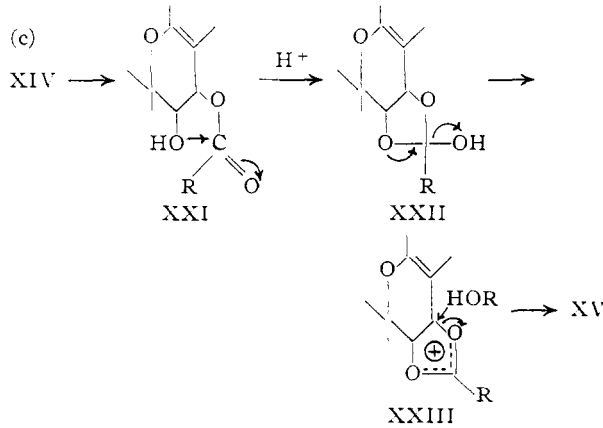
XVII

trans-2-acetoxycyclohexyl *p*-toluenesulfonate (XVIII), demonstrated the absence of any internal rearrangement phenomenon during solvolysis, thereby vitiating the case for the process (XIX → XVIII) or (XVIII ⇄ XX); the absence of this type of change[17] was considered as due to "the


XVIII XX XIX

special nature of the neighboring acetoxy group."[18] Application of these findings to the case under consideration seems direct and convincing; we therefore consider mechanisms a and b inoperative.

Still a third route can be envisioned for the hypothetical change XIV → XV. In (c), a selective removal of acetyl from XIV yields initially the monoester XXI which then cyclizes, as the first stage in an acyl migration, to XXII. The latter intermediate could advance to the isomeric mono-



XXI XXII XXIII

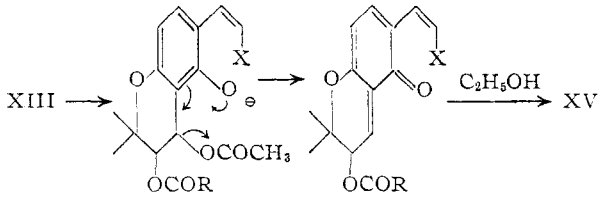
ester, which in itself would be incapable of yielding an ether, or it could undergo alcoholysis, possibly *via* the mesomeric cation XXIII, and finally afford structure XV. The solvolysis rate data on samidin can be marshalled so as to exclude convincingly path c. Bearing in mind that the ester group in XV and the acetate substituent in XIV would possess the same steric environment, one concludes that the rate difference in hydrolysis of both ester functions should approximate that between simple esters of acetic acid and senecioic acid. Although this specific information apparently has not been recorded, rough calculations, using accessible rate data,[18] show that ethyl acetate and ethyl crotonate differ in basic hydrolysis rate by a factor of only about ten.

The difference between the cases XIV and XV should be of the same order of magnitude. However, this expectation is contrary to fact, in that samidin, in the presence of excess alcoholic alkali, consumes

(15) A. G. Davies and J. Kenyon, *Quart. Revs.*, **9**, 203 (1955).
(16) S. Winstein and R. Heck, THIS JOURNAL, **74**, 5584 (1952).
(17) The reality of ion pairs in other cases where either tosylate or acetate is the leaving group has been demonstrated (D. J. Cram, *ibid.*, **74**, 2129 (1952); S. Winstein and K. C. Schreiber, *ibid.*, **74**, 2165 (1952)).
(18) P. Heinanen, *Ann. Acad. Sci. Fennicae*, Ser. A, II, Chemica, No. 9, 3 (1943).

at room temperature almost two moles of base in six minutes without further uptake after 4 hr. These are the conditions used to prepare XV; therefore, the two moles are used to discharge acetoxyl and to open the lactone ring, *the senecioate grouping being essentially inert under these conditions.* Consequently mechanism c too may be discarded, and structure XIII is at last vindicated.

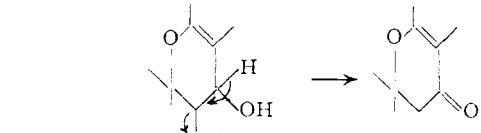
There remains the question of the actual solvolysis mechanism which leads to XV. Samidin was subjected to conditions comparable to those used in the normal solvolysis, except that, instead of alkali, such a quantity of salt was used as to ensure a medium of comparable ionic strength. The compound was recovered after such treatment. This simple experiment demonstrates that the alcoholysis is not an SN1 process, which is the typical one in benzyl ester O-alkyl cleavage.[15] Although O-alkyl cleavage by the SN2 process is not unknown,[15] we prefer the route wherein samidin solvolyzes by a process involving initial opening of the lactone ring by base, followed by a rapid, SN1 type of acetoxyl expulsion triggered by the neighboring phenoxide anion. Addition of solvent to the enone intermediate XXIV (or to the carbonium ion resulting from protonation of XXIV) completes the reaction.[19]



X = $COO^{\ominus}$ or $COOC_2H_5$ XXIV

Of the reactions described, only one more requires special comment: formation of 3-methyl-β-resorcylic acid in the basic fusion of samidin, an unusual result in that *a partially oxidized carbon atom is converted to methyl.*

Base-catalyzed dehydration with β-elimination of hydroxyl (XXV → XXVI) might have been anticipated; however, the observed behavior is not without precedent,[20] and the process may be interpreted mechanistically along the following lines



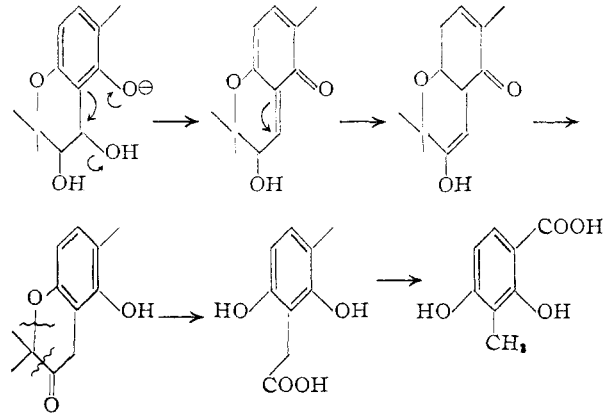


TABLE II

EFFECT ON RATE OF FLOW THROUGH ISOLATED RABBIT HEART[a]

Compound	Concentration	Rate of flow Control, ml./2 min.	Sample ml./2 min.	Increase, %	Relative potency
Khellin	1:60,000	26.5	37	40	1
Visnagan	1:150,000	30.7	43	41	2.5
Khellin	1:30,000	28.0	39.7	41.8	1
Visnadin	1:60,000	21.0	49.1	131.6	
Visnadin	1:300,000	28.5	39.7	39.2	10
Khellin	1:30,000	26.5	37.0	39.6	1
Visnadin Mother Liquors	1:240,000	23.5	30.8	31.2	App. 8
Khellin	1:15,000	24.7	45.6	84.6	1
Dihydrosamidin	1:75,000	24.7	45.0	82.2	5
Khellin	1:15,000	29.2	47.7	63.2	1
Papaverine HCl	1:75,000	27.2	37.2	36.7	2.5

[a] Hazleton Laboratories, Falls Church, Virginia.

Pharmacology.—Visnadin and dihydrosamidin were compared with khellin and papaverine hydrochloride for vasodilatory activity (Table II). Gomes,[21] who confirmed the isolation of crystalline visnadin, found it to exert an antispasmodic action about three times that of papaverine on the barium chloride- and acetylcholine-induced spasm of the isolated rabbit gut. A similar activity was found against histamine-induced spasm of guinea pig intestine.

The non-crystallizable mother liquors remaining after removal of the described substances still contained considerable activity. Their ultraviolet spectra, which were identical with those of the compounds described above, show that this activity probably was derived from material of similar chemical structure. These mother liquors are being investigated further.

Experimental[22]

Purification of Visnagan.—About 10 lb. of visnagan prepared according to Samaan[3] was mixed with 6 gal. of pentane and this solution extracted repeatedly with 80% alcohol, using 6, 2 and 2 gallons, respectively. The alcoholic layers were separated, washed once with pentane and concentrated *in vacuo*. The residue was diluted with 2 gal. of water and extracted with ether. The combined ether extracts were dried over anhydrous sodium sulfate, filtered and gaseous hydrochloric acid passed through the solution

(19) The arguments leading to structure XIII render untenable the structures of samidin, dihydrosamidin and visnadin proposed earlier by E. Smith, N. Hosansky and W. G. Bywater.[5b] The stereochemistry of XIII is not known but is arbitrarily designated on occasion as *trans* (*e.g.*, XVI).

(20) D. Sontag, *Ann. chim.*, [11] **1**, 359 (1934), reported that stilbene glycol is converted in part by potassium hydroxide at 250° to 1,2-diphenylethane; L. Palfray, S. Sabetay and D. Sontag, *Compt. rend.*, **193**, 941 (1931), state that styrene glycol apparently yields some toluene under similar conditions.

(21) F. P. Gomes, *Compt. rend. soc. biol.*, **147**, 1836 (1953).

(22) Microanalyses and molecular weight determination by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. The rotational and ultraviolet absorption measurements were run in U.S.P. alcohol. Intensities are expressed as log ε.

under cooling as long as oxonium salts precipitated. The ether solution was filtered, washed acid-free with sodium carbonate solution, then washed with water, dried over anhydrous sodium sulfate and concentrated; 160 g. of clear brown oil remained. This fraction had an optical activity of +22°.

Chromatography of Visnagan.—Seven pounds of the refined oil was diluted with 5 lb. of ether and mixed into 7 lb. of Filter-cel. The mix was air-dried, put through a screen and then added to a column containing 127 lb. of silica (J. F. Smith) which had previously been covered with pentane. The column was developed and cut, according to the following scheme: (1) Fifteen gallons of pentane which eluted 690 g. of oil not further investigated; (2) fifty gallons of an ether–pentane mixture containing 10 to 20% ether. This eluted 59 g. of a non-crystalline oil with an optical activity of +55°. The oil, rubbed up with alcohol, gave 24 g. (RI-860) of crystals which, after repeated recrystallizations from alcohol, melted at 285–287°, $[\alpha]_D$ +69°. This fraction was followed by a bright yellow eluate which on concentration gave 113 g. of oil which, when rubbed up with ether, gave 10 g. of bright yellow crystals ("yellow body") melting, after repeated crystallizations from methanol, at 176–178°, λ_{max} 293 mμ (E^{1}_{1} 1580). (3) Thirty gallons of ether pentane (1:3). The eluate began to show the typical ultraviolet absorption spectrum of the dihydrofurocoumarins; an absorption minimum at 268 mμ and an absorption maximum at 320 mμ. At the same time, the absorption was taken at 220 mμ. As long as the eluate showed an absorption ratio of 322 mμ:222 mμ of 1–1.7:1 and 322 mμ:262 mμ of 2–4:1, it was collected for the preparation of *visnadin*. A total throughput of 75 gal. was collected, concentrated to 5 gal. and allowed to crystallize for a week in the ice-box. There were thus obtained 236 g. of crude visnadin, melting at 75–78° and having an optical activity of +12°, and 190 g. of oil with an optical activity of +10°. (4) The next fraction was collected until the ratio 322 mμ:220 mμ had risen to about 6:1. This fraction eluted *samidin*. It required about 30 gal. of ether–pentane (1:3) and 40 gal. of ether–pentane (3:7). The combined eluates were concentrated to 6 gal. and allowed to crystallize; 320 g. of crude samidin, with a melting point 127–135° and an optical activity of +20°, and 220 g. of a non-crystallizable oil were recovered from this fraction. (5) At this point the ultraviolet absorption spectrum of the eluate showed a distinct shift of the minimum from 262 to 270 mμ and of the maximum from 320 to 300 mμ. Further elution with ether–pentane (1:1) resulted in the isolation of *visamminol*. Fifty gallons of eluate was needed for total removal and gave, on concentrating to 5 gal., 215 g. of visamminol and 200 g. of non-crystallizable oil. A further increase in the ether concentration resulted in the elution of 110 g. of *visnagin* and an additional 270 g. of oil.

Visnadin.—The crude fraction (236 g.) was dissolved in 500 ml. of ether, charcoaled, 600 ml. of hexane was added; the solution was concentrated to 600 ml. and allowed to crystallize. The 200 g. of visnadin so obtained was once more crystallized and gave 160 g. of white needles, m.p. 84–86°, $[\alpha]_D$ +9° (*c* 2.0) and +38° (*c* 1.0 in dioxane). In a volatile acid determination, 46.0 mg. yielded an amount of acid equivalent to 11.29 ml. of 0.02 *N* sodium hydroxide; calcd. for two acids, 11.75 ml.

Anal. Calcd. for $C_{21}H_{24}O_7$: C, 64.95; H, 6.23. Found: C, 65.04; H, 6.19.

Dihydrosamidin.—The mother liquors of the visnadin recrystallizations were concentrated to half-volume and an additional small amount of impure visnadin was isolated. They were then taken down to dryness and rubbed up with 20 ml. of ether and allowed to crystallize in the ice-box; 6 g. of crystals was obtained which, when recrystallized from 90% methanol, gave 5 g. of pyramids, m.p. 117–119°, $[\alpha]_D$ +19° (*c* 1.0) and +63° (*c* 1.0 in dioxane). On volatile acid determination, 84.8 mg. used 22.08 ml. of 0.02 *N* sodium hydroxide; calcd. for two acids 21.86 ml.

Anal. Calcd. for $C_{21}H_{24}O_7$: C, 64.95; H, 6.23. Found: C, 64.91; H, 6.09.

Samidin.—The 320 g. of crude samidin was repeatedly crystallized from alcohol to give 250 g. of pure material as cubes, m.p. 135–137°, $[\alpha]_D$ +26° (*c* 1.0) and +100° (*c* 1.0 in dioxane). In a volatile acid determination, 40.6 mg. used 9.99 ml. of 0.02 *N* sodium hydroxide; calcd. for two acids 10.51 ml.

Anal. Calcd. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 65.21; H, 5.53.

Hydrogenation of Samidin.—Twenty grams of samidin was dissolved in 200 ml. of alcohol and hydrogenated with 200 mg. of platinum oxide. After 1 mole of hydrogen was consumed, the hydrogenation was interrupted, the catalyst filtered off, and the alcoholic solution concentrated to 20 ml., 12 g. of substance crystallized out which, on recrystallization from 90% methanol, melted at 115–117°, $[\alpha]_D$ +16° (*c* 1.0); no mixed m.p. depression with natural dihydrosamidin.

Room Temperature Hydrolysis. Titration Experiments.—Samidin (59.8 mg., 0.154 mmole) and visnadin (45.6 mg., 0.117 mmole), respectively, were dissolved in 10.0 ml. of 0.25 *N* sodium hydroxide in alcohol and after time, *t*, at room temperature a 1.0-ml. aliquot was removed, diluted with 10 ml. of CO_2-free water and titrated with 0.025 *N* sulfuric acid, using phenolphthalein as indicator. A blank consisting of the alcoholic alkaline solution was titrated at the same time. After *t* = 6, 80 and 230 min., the moles of alkali consumed were for samidin 1.73, 2.23 and 2.19, and for visnadin 1.78, 2.10 and 2.11. The remainder of the solutions were then refluxed for 1 hr., cooled and again titrated as above. The total mole uptake for samidin was 3.06, for visnadin 3.25.

Samidin (5 g.) was dissolved in 45 ml. of ethanol and 45 ml. of 1 *N* sodium hydroxide in ethanol was added. The solution was allowed to stand at room temperature and its rotation taken every 10 min. The rotation increased for about 45 min., after which the solution was diluted with 1 l. of water, acidified with sulfuric acid and extracted with ether. The ether solution was backwashed with dilute sodium carbonate solution, dried and concentrated. The residue was twice crystallized from 80% methanol and gave 3 g. of XV, m.p. 91–93°, $[\alpha]_D$ +99° (*c* 0.9); λ_{max} 326 mμ (4.16), $\lambda_{min.}$ 264 mμ (3.42). On volatile acid determination 47.7 mg. of substance used 5.92 ml. of 0.02 *N* sodium hydroxide; calcd. for 1 acid 6.4 ml.

Anal. Calcd. for $C_{21}H_{24}O_6$ (372.4): C, 67.73; H, 6.50. Found: C, 67.81; H, 6.51.

Isolation of the Acid.—The sodium carbonate wash of the ether was combined with the original aqueous solution and concentrated *in vacuo* on the alkaline side. It was then acidified with 5 *N* sulfuric acid and steam distilled. Ten 100-ml. fractions were collected, neutralized and concentrated *in vacuo* to 50 ml. A 5-ml. aliquot was taken for the preparation of the *p*-phenylphenylacyl ester which was obtained with a m.p. 110–111°, no mixed melting point depression with the ester of acetic acid. A second 5-ml. aliquot was taken for the preparation of the *p*-bromophenacyl ester. Crystallized from hexane and recrystallized from aqueous alcohol, the ester melted at 84–86° and showed no mixed m.p. depression with authentic ester prepared from acetic acid.

Hot Ethanolic Alkaline Hydrolysis.—Twenty-five grams of samidin in ethanol was refluxed with 165 ml. of 2 *N* alcoholic sodium hydroxide for 1.5 hr. The orange colored solution was cooled, diluted with water, concentrated *in vacuo* to remove alcohol, acidified with concentrated sulfuric acid and extracted with ether. The ether was backwashed with sodium bicarbonate solution, dried and concentrated. Pentane was added to the concentrated solution to slight turbidity, whereupon crystallization occurred. The 7.4 g. (40% of theory) of III so obtained was repeatedly recrystallized from an ether–pentane mixture; m.p. 161–162°; $[\alpha]_D$ −59° (*c* 0.9), λ_{max} 325 mμ (4.15), $\lambda_{min.}$ 266 mμ (3.18). The same compound was obtained when either XV or visnadin or dihydrosamidin was used as starting material.

Anal. Calcd. for $C_{16}H_{18}O_5$ (290.3): C, 66.19; H, 6.25; OC_2H_5, 15.52. Found: C, 66.20; H, 6.11; OC_2H_5, 16.00.

Isolation of Acid.—The sodium bicarbonate wash of the ether solution was combined with the original water solution and concentrated on the alkaline side *in vacuo*, acidified and extracted thoroughly with ether. The ether was concentrated, after drying, to give a yellow, oily residue which distilled at 20 mm. at 160–180°. The distillate crystallized and was redistilled at atmospheric pressure. The fraction coming over at 190–193° was recrystallized from water; m.p. 68–69° (lit.[23] for senecioic acid 68–70°).

The *p*-toluidide was prepared over the acid chloride; m.p. 107–108° (lit.[23] 106–107°). *Anal.* Calcd. for C_{12}-

(23) R. B. Wagner, THIS JOURNAL, 71, 3214 (1949).

$H_{15}ON$: C, 76.15; H, 7.99; N, 7.40. Found: C, 76.20; H, 7.88; N, 7.26.

The *p*-phenylphenacyl ester was prepared in the usual way; m.p. 144.5–146°. *Anal.* Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.17. Found: C, 77.54; H, 5.76.

On hydrogenation with platinum oxide, the acid consumed 1 mole equivalent of hydrogen. The *p*-phenylphenacyl ester of the hydrogenated acid had a m.p. 76–77°; no depression on admixture with the ester of isovaleric acid. *Anal.* Calcd. for $C_{19}H_{20}O_3$: C, 76.98; H, 6.87. Found: C, 77.00; H, 6.49.

The acid isolated in the same manner from visnadin gave a *p*-phenylphenacyl ester melting at 69–70° (lit. for 1-α-methylbutyric acid, 70–71°)[24] and a *p*-bromphenacyl ester, m.p. 54–55° (lit.[24] 55°).

Acetate of III.—On acetylation with acetic acid anhydride in pyridine, 392 mg. of III gave, after crystallization from aqueous methanol, 385 mg. of monoacetate, m.p. 170–172°, $[\alpha]_D$ −40.8° (*c* 0.6%), λ_{max} 325 mμ (4.16), λ_{min}. 264 mμ (3.22).

Anal. Calcd. for $C_{18}H_{20}O_6$ (332.3): C, 65.05; H, 6.07. Found: C, 65.23; H, 6.03; mol. wt. by Rast, 332.

Oxidation of III.—III (1.16 g.) was dissolved with slight warming in 1% sodium hydroxide solution. To the cooled solution a saturated solution of permanganate was added until the violet color persisted. After acidification the solution was steam distilled and the distillate precipitated with *p*-nitrophenylhydrazine. The hydrazone obtained (240 mg.) melted, after recrystallization, at 147–149°; no depression on admixture with *p*-nitrophenylhydrazone prepared from acetone.

Hydrogenation and Oxidation of III.—III (4 g.) was hydrogenated in 40 ml. of glacial acetic acid with 400 ml. of palladium-on-charcoal. The uptake was very slow, one mole equivalent being taken up after 9 hr. The catalyst was filtered off, the filtrate diluted with water and extracted with ether. The ether was backwashed with sodium carbonate solution, dried and concentrated. The residue was crystallized twice from ethyl acetate–hexane (1:2) to give 3.56 g. of the reduced product, m.p. 125–127°, $[\alpha]_D$ −11°, dissolving colorless in alcoholic alkali; λ_{max} 287 mμ (3.36), λ_{min}. 261 mμ (2.81).

One gram of this substance was dissolved in ice-cold fuming nitric acid (d. 1.4) and allowed to stand at room temperature for 12 hr. The yellow solution was then heated on the steam-bath for 8 hr. and taken down to dryness. The residue was dissolved in dilute ammonia, the *p*H adjusted to 5.5 and the oxalic acid precipitated from the boiling hot solution with calcium chloride. The precipitate was filtered off and the filtrate concentrated *in vacuo*, acidified and extracted in a liquid–liquid extractor with ether. The ether deposited crystals on concentrating, which were collected and then sublimed *in vacuo*. The sublimate melted at 187–188° and did not give a m.p. depression on admixture with succinic acid; 25.6 mg. of substance used 21.2 ml. of 0.02 *N* sodium hydroxide; calcd. for succinic acid, 21.6 ml.

Hot Methanolic Alkaline Hydrolysis.—Twenty grams of samidin in 85 ml. of methanol was refluxed with 85 ml. of 2 *N* methanolic sodium hydroxide for 1.5 hr. The solution was then concentrated *in vacuo*, diluted with water, acidified and extracted with ether. The ether was backwashed with dilute sodium carbonate solution, water, dried and concentrated. The residue was crystallized from ether–pentane to give 6.48 g. (44%) of IV, m.p. 158–159°, $[\alpha]_D$ +14° (*c* 1.04), λ_{max} 328 mμ (4.18), λ_{min}. 267 mμ (3.21).

Anal. Calcd. for $C_{15}H_{16}O_5$ (276.3): C, 65.19; H, 5.84; OCH_3, 11.23. Found: C, 65.46; H, 5.76; OCH_3, 11.17.

On acetylation, 400 mg. of IV gave, after recrystallization from methanol, 318 mg. of the monoacetate, m.p. 147–149°, $[\alpha]_D$ +18.4° (*c* 1.29), λ_{max} 324 mμ (4.17), λ_{min}. 265 mμ (3.19).

Anal. Calcd. for $C_{17}H_{18}O_6$ (318.31): C, 64.14; H, 5.70. Found: C, 64.12; H, 5.83.

The same alcohol and acetate were obtained when the hydrolysis was conducted with methanolic hydrochloric acid.

Preparation of Diol V.—Eight hundred and eighty-four mg. of III was dissolved in 5 ml. of hydriodic acid (d. 1.70). After about 20 min., crystalline material began to separate out and was collected after standing 1 hr. After several crystallizations from acetone, 400 mg. of V was obtained, m.p. 180–182°, $[\alpha]_D$ +13° (*c* 0.7), λ_{max} 328 mμ (4.17), λ_{min}. 265 mμ (3.16).

Anal. Calcd. for $C_{14}H_{14}O_5$ (262.3): C, 64.11; H, 5.38. Found: C, 64.16; H, 5.37.

V, acetylated in the usual way, gave after recrystallization from methanol, the diacetate, m.p. 161–163°, $[\alpha]_D$ −27° (*c* 0.69), λ_{max} 323 mμ (4.14), λ_{min}. 263 mμ (3.21).

Anal. Calcd. for $C_{18}H_{18}O_7$ (346.3): C, 62.42; H, 5.24. Found: C, 62.54; H, 5.07; mol. wt., 344.8 (Rast).

The same substance was obtained when samidin was refluxed with aqueous sodium hydroxide. From 20 g., 7.7 g. of oil was recovered, which was chromatographed over alumina. The eluate obtained by developing with ether crystallized to give, after repeated crystallization from methanol, 2 g. of V, m.p. 183–184°, $[\alpha]_D$ +11°, no mixed m.p. depression with V prepared from III.

Anal. Found: C, 64.07; H, 5.40.

Dehydration of V.—V (200 mg.) was refluxed with 0.5 ml. of acetic acid and 0.5 ml. of concd. hydrobromic acid for 15 min. The solution was cooled, diluted with water and extracted into ether. The ether, after washing with sodium bicarbonate and water, was dried, evaporated to dryness and the residue crystallized from ethyl acetate. The product XII, after several crystallizations, melted at 156–157°, $[\alpha]_D$ 0 (*c* 2.0), λ_{max} 321 mμ (4.11), λ_{min}. 263 mμ (3.45).

Anal. Calcd. for $C_{14}H_{12}O_4$: C, 68.86; H, 4.95. Found: C, 68.54; H, 4.95.

Alkaline Fusion of Samidin.—Three and two-tenths grams of samidin was added in small portions to a sodium hydroxide–potassium hydroxide melt kept at 150–160°. The temperature was then raised slowly to 230° and kept at that temperature until the melt cleared. After cooling, the melt was dissolved in water, filtered, chilled in ice and acidified with concentrated hydrochloric acid. Insoluble flocculent material was filtered off and the filtrate extracted with ether. The ether gave, on concentration, 1.5 g. of amorphous residue.

This material was chromatographed on 20 g. of silica with benzene. The first benzene eluates gave, on concentration, 910 mg. of crystalline material, m.p. 213–214° with evolution of gas, λ_{max} 262 mμ (4.14), λ 297 mμ (3.65). Its infrared spectrum was indistinguishable from that of 3-methyl-β-resorcylic acid.

Anal. Calcd. for $C_8H_8O_4$ (168.1): C, 57.14; H, 4.80. Found: C, 56.92; H, 5.11; mol. wt. (Rast), 163.3.

Preparation of 2-Methylresorcinol.—One hundred and sixteen mg. of the acid was melted in a sealed tube at 220° and kept at that temperature until gas evolution stopped. The cooled contents were dissolved in benzene and chromatographed on silica. From the first benzene eluates 76 mg. of crystals was isolated, sublimed in high vacuum at 60–70° to give pure material melting at 117–119°. Its infrared spectrum was identical with that of authentic 2-methylresorcinol.

Anal. Calcd. for $C_7H_8O_2$ (124.1): C, 67.73; H, 6.50. Found: C, 68.00; H, 6.42; mol. wt. (Rast), 131.

Periodate Oxidation of V.—Twelve and nine-tenths mg. (0.049 mmole) of diol V was dissolved in 5.0 ml. of 0.098 molar periodic acid in 80% methanol (distilled from zinc–sodium hydroxide). The analysis was standard, excess arsenite being titrated by 0.00946 molar iodine.

Time, hr.	HIO_4 consumed, mole
0.17	0.35
0.50	0.40
2.50	0.65
12.50	1.00
17.00	0.98
21.00	1.00

Dimedon was employed to detect any volatile aldehyde. However, apart from a small amount of amorphous material, no derivative was obtained.[25]

MADISON, WISCONSIN

(24) E. H. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1941, p. 191.

(25) The authors are grateful to Dr. John Dyer for these determinations.