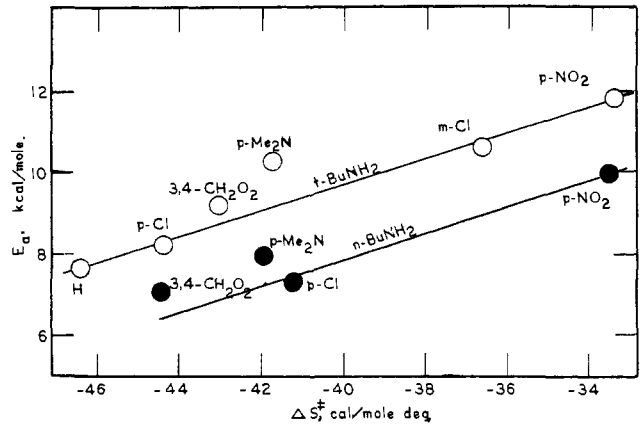


Fig. 2.—Enthalpy–entropy plots for substituted benzaldehydes with *n*- and *t*-butylamine.

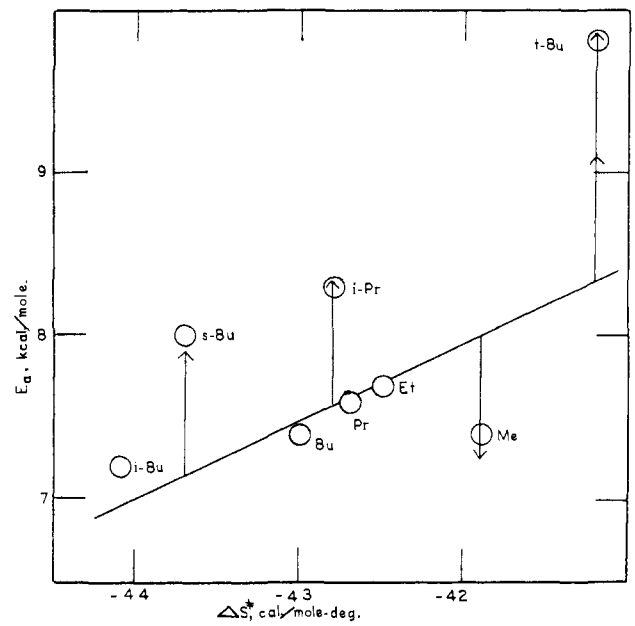


Fig. 3.—Enthalpy–entropy plot for piperonal with primary alkylamines in terms of deviation from a single line caused by α-substitution.

In the enthalpy–entropy diagram[16] in Fig. 2, a straight line is drawn through four points of the *t*-butyl series, the slope corresponding to an isokinetic temperature of 316°K. The points for piperonal and *p*-dimethylaminobenzaldehyde are above the line, possibly because of the strong resonance interaction associated with highly negative σ^+ values. If a line of the same slope is passed through the *p*-nitrobenzaldehyde point in the *n*-butylamine series the few available points show similar behavior.

Another extrathermodynamic relationship in Schiff base formation is seen in Fig. 3, an enthalpy–entropy plot of our earlier data[8] for the reaction of piperonal with all the primary aliphatic amines through $C_4H_9NH_2$. If a line of slope 415° is drawn through the point for ethylamine, the points for the other amines are displaced upward from the line by approximately $0.75(n - 1)$ kcal., where n is the number of methyl groups attached to the carbon atom bearing the amino group. The data are insufficient to evaluate the effect of chain branching in the β-position as in isobutylamine.

Acknowledgment.—We are grateful for Grants G-4242 and G-13292 from the National Science Foundation. The least-squares solutions were computed by Dr. Hermon Parker and his staff.

(16) Reference 13a, Chapter 9.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA, AND LABORATORY OF METABOLISM, NATIONAL HEART INSTITUTE, BETHESDA, MARYLAND]

The Structure of Amaryllisine

BY A. L. BURLINGAME,[1a] H. M. FALES,[1b] AND R. J. HIGHET[1b]

RECEIVED JUNE 18, 1964

Amaryllisine has been shown to possess structure III on the basis of close correspondence of infrared, nuclear magnetic resonance, and mass spectra to those of alkaloids of known structure. The failure of combustion analysis is noted.

Although all alkaloids of the *Amaryllidaceae* contain oxygenated aromatic systems, free phenols are seldom isolated.[2] It was therefore of some interest when chromatography of the alkaloid extract from *Brunsvigia rosea* (*Amaryllis belladonna*) over alumina produced a phenolic compound, m.p. 255–258°, which was eluted by chloroform containing ethyl alcohol. Combustion analysis and neutral equivalent of this phenol corresponded to the formula $C_{17}H_{21}NO_4$, and analysis showed three methoxyl groups and no N–CH_3. The compound, which was named amaryllisine, possessed a small positive rotation, $[\alpha]^{24}D$ 2.4°, and ultraviolet absorption suitable to a phenol (λ_{max} 283 mμ (ε 5900)) and shifted by addition of base to λ 252 mμ (ε 6600), 297 mμ (ε 4080). The infrared spectrum at high dilution in carbon tetrachloride showed the presence of a hydrogen-bonded hydroxyl (3547 cm.$^{-1}$), olefinic hydrogen (3032 cm.$^{-1}$), the aromatic system (1615 and 1590 cm.$^{-1}$), and ether linkages (1095, 1068 cm.$^{-1}$).[3] The methylenedioxy group, which occurs frequently in this family of alkaloids, was absent, for its presence would have been unmistakably revealed by an additional peak near 965 cm.$^{-1}$.[4] The occurrence of three methoxyl groups in the formula $C_{17}H_{21}NO_4$ allows fourteen carbon atoms and the nitrogen atom to form the basic ring system. However, with a single exception,[5a]

(1) (a) University of California; (b) National Heart Institute.

(2) For recent reviews of the alkaloids of this family, see W. C. Wildman in "The Alkaloids," Vol. VI, R. H. Manske, Ed., Academic Press, Inc., New York, N. Y., 1960, p. 289; H.-G. Boit, "Ergebnisse der Alkaloid-Chemie bis 1960," Academie-Verlag, Berlin, 1961, p. 410.

(3) *Cf.* L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958.

(4) L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, *Anal. Chem.*, **29**, 904 (1957).

(5) (a) Ismine, 6-(2-methylaminophenyl)piperonyl alcohol, is thought to be a natural degradation product from the series haemanthidine–tazettine–haemanthamine (R. J. Highet, *J. Org. Chem.*, **26**, 4767 (1961)). (b) For a review of the position of norbelladine in the biosynthesis of *Amaryllidaceae* alkaloids, see A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 189 (1963).

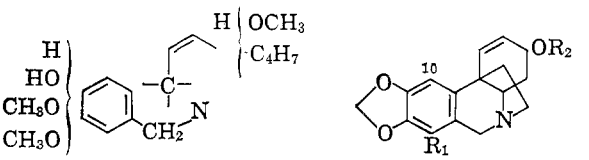
alkaloids of this family possess ring systems of fifteen carbon atoms, derived from the parent system of the biogenetic precursor norbelladine.[5b] The possibility that the alkaloid represents a novel variant on this system prompted further investigation.

Treatment of amaryllisine with diazomethane produced a methyl ether, m.p. 99–100°, $[\alpha]^{24}D$ −16°, which formed a crystalline perchlorate, m.p. 236–237°, the analysis of which was consistent with the anticipated formula $C_{18}H_{23}NO_4 \cdot HClO_4$. The alkaloid absorbed a single mole of hydrogen to form a dihydro derivative, m.p. 243–245°, $[\alpha]^{24}D$ −11°, with an analysis consistent with $C_{17}H_{23}NO_4$; the unaltered ultraviolet absorption shows that the double bond is not conjugated with the aromatic ring. As the molecule contains a single double bond, it evidently retains a tetracyclic system, including one aromatic ring, in common with other alkaloids of the *Amaryllidaceae*. Refluxing the alkaloid with acetic anhydride produced a basic acetate, m.p. 183–185°, with an analysis consistent with $C_{19}H_{23}NO_5$; the infrared spectrum of this compound shows no OH nor NH peaks, but the anticipated ester peaks at 1765 and 1230 cm.$^{-1}$. The nitrogen was thus demonstrated to be tertiary. In each case, it is emphasized that both combustion analyses and neutral equivalents conformed to the empirical formula consistent with a tetracyclic ring system including fourteen carbon atoms.[6] The theoretical composition of the fifteen-carbon ring system anticipated by analogy with other alkaloids of the family differed appreciably from the observed results in each case.[7]

The infrared spectrum failed to reveal any abnormal variation from familiar amaryllis alkaloids and resembled closely those of the crinine group of alkaloids in lacking the bands near 2500 cm.$^{-1}$, first reported in quinolizidine systems,[8] and apparent in the spectra of alkaloids related to lycorine. The presence of a cyclopentene could be excluded, for the olefinic protons were represented by a peak at 3032 cm.$^{-1}$.[9]

The study of the nuclear magnetic resonance spectrum, to be discussed below, also failed to reveal an aberrant ring, and it appeared impossible, at this point, to formulate a structure that would conform to all of the information in hand. Furthermore, the paucity of material precluded extensive degradative studies. This problem was eventually solved by mass spectrometric molecular weight determinations on the alkaloid and its dihydro derivative, which display intense molecular ion peaks at *m/e* 317 and 319, respectively. Subsequently, the empirical composition of amaryllisine was confirmed as $C_{18}H_{23}NO_4$ [317] by accurate mass measurements of its molecular ion.[10] It was apparent that the analytical methods previously reliable in this family had given erroneous, if consistent, results.

Study of the nuclear magnetic resonance spectrum now made it possible to assign the structure of amaryllisine. The appearance of strong single peaks at τ 6.13, 6.20, and 6.63 confirmed the presence of three methoxyl groups, two aromatic and the third attached to a saturated carbon atom. The single remaining aromatic proton appeared as a single peak at τ 3.28. A quartet corresponding to 2-protons appeared with chemical shifts of τ 5.44 and 6.08, J = 17 c.p.s., which is similar to that in spectra of other amaryllis alkaloids[11] and demonstrates the presence of the nonequivalent protons of the benzylic methylene group of the tetrahydroisoquinoline system. Finally, the peaks from the olefinic protons appeared as two groups, that at lower field as a doublet centered at τ 3.37, separated by 9 c.p.s., while that at higher field appeared as a quartet, resulting from the splitting of a doublet symmetrical to that at τ 3.37 by a further coupling of 5 c.p.s. Thus, this system revealed the specific situation of I and, together with the other interpretable peaks, revealed the nature of substitution on fourteen of the eighteen carbon atoms of the molecule.



I IIa, $R_1 = R_2 = H$; b, $R_1 = OCH_3$; $R_2 = CH_3$

It is clear that the partial structures revealed by these studies can readily be assembled as a derivative of crinine (IIa). The validity of this inference was substantiated in the mass spectra of amaryllisine, Fig. 1, O-methylamaryllisine, Fig. 2, and dihydroamaryllisine, Fig. 4, *via* comparison with buphanidrine (IIb), Fig. 3, and dihydrobuphanidrine (IIb, no double bond), Fig. 5. From direct comparison it is apparent that amaryllisine and buphanidrine differ in the high mass region by a constant increment of 2 m.u. (mass units) [$C_2H_2O_3$ (74) *vs.* $C_2H_4O_3$ (76)]. Thus, the structural identity of the alicyclic moiety with the crinine-type carbon skeleton is confirmed, and the applicability of the mass spectrometric shift technique to the 5,10b-ethano-3,4,4a,5,6,10b-hexahydrophenanthridine skeleton is demonstrated.[12] Similarly, the fragmentation patterns of dihydroamaryllisine and dihydrobuphanidrine, Fig. 4 and 5, are consistent with their common heterocyclic system and provide further confirmation, especially since the fragmentation pattern

(6) These analyses were performed by J. F. Alicino of Metuchen, N. J., whose dependable analyses have contributed greatly to the chemistry of alkaloids in this family. The scant quantities available made repeated analyses impossible, but the consistent results from different materials show clearly that these aberrations (*v.i.*) are a property of the molecule in hand.

(7) In particular, the observed analyses are consistently about 1% lower in carbon than those required by the fifteen-carbon system.

(8) F. Bohlmann, *Chem. Ber.*, **91**, 2157 (1958).

(9) The C–H stretching peaks of simple cyclopentenes are known to occur near 3050 cm.$^{-1}$ (*cf.* W. H. Tallent and I. J. Siewers, *Anal. Chem.*, **28**, 953 (1956)). Typical observations on fused-ring systems include dicyclopentadiene, 3057, 3050 cm.$^{-1}$; norbornylcarbinol (*endo–exo* mixture), 3062; norbornylene, 3061; bicyclo[2,2,1]heptadiene-2,5, 3070; *endo-cis*-bicyclo[2,2,1]5-heptene-2,3-dicarboxylic anhydride, 3070; tenulin, 3080, 3069. We are indebted to Professor P. de Mayo for the gift of tenulin.

(10) Accurate mass measurement of the molecular ion was obtained from a photographic plate (CEC 21-110 mass spectrometer) using perfluorokerosene as a mass standard. The measured value was 317.1602; $C_{18}H_{23}NO_4$ requires 317.1627.

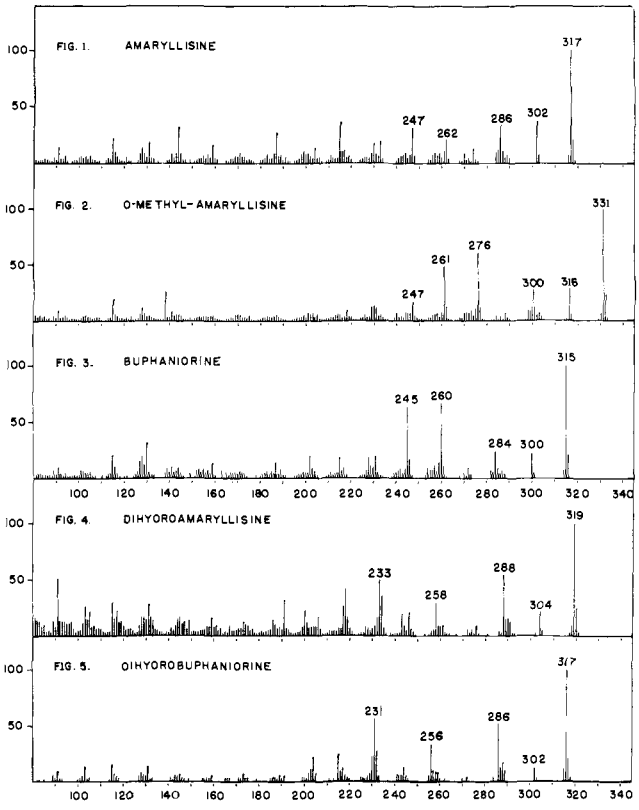
(11) H. A. Lloyd, E. A. Kielar, R. J. Highet, S. Uyeo, H. M. Fales, and W. C. Wildman, *J. Org. Chem.*, **27**, 373 (1963).

(12) (a) See footnote 10a in: K. Biemann, M. Friedmann-Spiteller, and G. Spiteller, *J. Am. Chem. Soc.*, **85**, 631 (1963). (b) The validity of the "mass spectrometric shift technique" has been demonstrated most conclusively recently by accurate mass measurement with subsequent comparison and verification of the empirical formulas of all major ions in the high-resolution mass spectra of ajmalidine and vomalidine; *cf.* K. Biemann, P. Bommer, A. L. Burlingame, and W. J. McMurry, *Tetrahedron Letters*, **No. 28**, 1969 (1963). (c) A review of the application of mass spectrometry in the elucidation of alkaloidal structures may be found in K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962. Chapter 8; H. Budzikiewicz, C. Djerassi, and D. W. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. I, Holden-Day, San Francisco, Calif., 1964, Chapter 3.

TABLE I
ANALYTICAL DATA

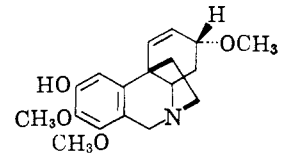
Compound		Calcd.	Found
Amaryllisine	$C_{17}H_{21}NO_4$ (303.3):	C, 67.31; H, 6.98; $(OCH_3)_3$, 30.69	C, 67.35; H, 6.27; OCH_3, 29.72
	$C_{18}H_{21}NO_4$ (317.4):	C, 68.12; H, 7.31; $(OCH_3)_3$, 29.33	Neut. equiv., 306
Methyl ether $HClO_4$	$C_{18}H_{24}NO_8Cl$(417.8):	C, 51.74; H, 5.79; $(OCH_3)_4$, 29.71	C, 51.71; H, 5.80; CH_3O, 29.47
	$C_{19}H_{26}NO_8Cl$(431.9):	C, 52.84; H, 6.07; $(OCH_3)_4$, 28.72	Neut. equiv., 413
O-Acetylamaryllisine	$C_{19}H_{23}NO_5$ (345.4):	C, 66.07; H, 6.71	C, 66.22; H, 6.91
	$C_{20}H_{25}NO_5$ (359.4):	C, 66.83; H, 7.01	Neut. equiv., 345
Dihydroamaryllisine	$C_{17}H_{23}NO_4$ (305.4):	C, 66.86; H, 7.59	C, 66.84; H, 7.72
	$C_{18}H_{25}NO_4$ (319.4):	C, 67.69; H, 7.89	Neut. equiv., 303

of the saturated system is drastically different. A detailed study of the electron-impact-induced fragmentation of this series is currently in progress in our laboratory.[13]



Figures 1–5.

Demonstrating the ring system of amaryllisine left unresolved the order of substitution of the aromatic system. The lone aromatic proton was revealed by the n.m.r. spectrum to be at C-10, for the chemical shift (τ 3.28) is moved upfield (to τ 3.40) by conversion to the dihydro derivative. This characteristic change is a result of the deshielding of the C-10 proton by the nearby C-1–2 double bond of the parent alkaloids, which is absent in the dihydro derivatives.[11] Studies reported elsewhere[14] have revealed that changes in the chemical shifts of aromatic protons of certain phenols upon conversion to the phenol anion are characteristic of the position of the proton relative to the phenolic hydroxyl. The shift of 14 cycles upfield observed on conversion of amaryllisine to the anion and that of 13 cycles on the formation of the anion of the dihydro derivative are typical of a proton *ortho* to a phenol. Thus, structure III represents amaryllisine.



III

The optical rotatory dispersion of amaryllisine methyl ether shows a plain curve of increasing levorotation, suggesting that its absolute configuration is that of (−)-crinine as shown in III. The small rotation is characteristic of an axially oriented methoxyl at the C_3-position; the epimers show negative rotations several times as large.

Experimental[15]

Crude alkaloidal extracts from *Amaryllis belladonna L.* (*Brunsvigia rosea* (Lam.) Hannibal), were partitioned between chloroform and 3 *N* hydrochloric acid. The acid layer was made basic with sodium bicarbonate, extracted with chloroform, and evaporated to dryness. A solution of 8.40 g. of this fraction was chromatographed over 300 g. of Florisil. Elution with 4% ethanol in chloroform produced 470 mg. of crude amaryllisine. Elution with 5–10% ethanol yielded a mixture containing 540 mg. of ambelline and 1.75 g. of buphanamine which were later separated by crystallization from ethyl acetate. Ethanol (10–50%) eluted 1.953 g. of powelline and finally 50–100% ethanol yielded 530 mg. of crude nerbowdine. All of the alkaloids found above were compared with authentic materials by infrared, melting point, and mixture melting point.

Amaryllisine was recrystallized several times from methanol as prisms, m.p. 255–258° dec., $[\alpha]^{24}D$ +2.4°, $[\alpha]^{24}_{436}$ −6.6° (*c* 0.27); λ_{max}^{EtOH} 283 mμ (ε 5900); $\lambda_{max}^{NaOH-EtOH}$ 252 mμ (ε 6600), 297 mμ (ε 4080); $\nu_{max}^{CCl_4}$ 3547 cm.$^{-1}$ (*o*-alkoxyphenol). On being stirred with Pd–C, 4.96 mg. absorbed 0.42 ml. of hydrogen (calcd. 0.42 ml.).

The methyl ether was prepared by the action of diazomethane on a methanolic solution of amaryllisine. Recrystallized as fine prisms from cyclohexane, it melted at 99–100°, $[\alpha]^{24}D$ −16.4°, $[\alpha]^{24}_{500}$ −25.9°, $[\alpha]^{24}_{436}$ −41.3° $[\alpha]^{24}_{400}$ −59° (*c* 1.60); λ_{max}^{EtOH} 275 mμ (ε 1210), 282 mμ (ε 1270).

The methyl ether hydroperchlorate was recrystallized from water; m.p. 236–237°, $[\alpha]^{24}D$ −4.2°, $[\alpha]^{24}_{500}$ −7.9°, $[\alpha]^{24}_{436}$ −14.8°, $[\alpha]^{24}_{400}$ −23° (*c* 1.50).

O-Acetylamaryllisine was prepared by heating 40 mg. of the base with 0.5 ml. of acetic anhydride in a sealed tube for 40 min. The excess anhydride was distilled under reduced pressure and the residue sublimed to provide material, m.p. 181–182°. The

(13) A. L. Burlingame, R. W. Olsen, H. M. Fales, and R. J. Highet, *J. Am. Chem. Soc.*, to be published.

(14) (a) R. J. Highet and P. F. Highet, *J. Org. Chem.*, submitted for publication. (b) The methyl carbon atom of the 8-methoxy group may well be the precursor of the methylenedioxy group of buphanidrine (11b); see D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *Proc. Chem. Soc.*, 179 (1961).

(15) Melting points were observed on a Kofler microscope hot stage and are corrected. Rotations were measured in chloroform with a Rudolph photoelectric spectropolarimeter using a 2-dm. tube, and ultraviolet spectra were obtained in absolute ethanol solution on a Cary Model 11 MS recording spectrophotometer. Infrared spectra were recorded on either a Perkin–Elmer Model 21 or a Beckman IR-7 double-beam spectrophotometer in chloroform solution, unless otherwise noted; n.m.r. measurements were obtained on a Varian A-60 spectrometer, in deuteriochloroform solution, using tetramethylsilane (τ 10.0) as an internal standard; spectra of phenolic anions were observed in D_2O, 0.5 *N* with respect to NaOD, and relative to cyclohexane as an external standard (τ 8.88). Mass spectra were determined with a C.E.C. 21-103 C mass spectrometer equipped with a heated glass inlet system (200°). All mass spectra were obtained at ionizing voltage of 70 e.v. and ionizing current of 50 μa.

infrared spectrum shows peaks at 1765, 1230, and 1065 cm.$^{-1}$.

Dihydroamaryllisine was prepared by stirring an ethanol solution of 17 mg. of the alkaloid with 15 mg. of 10% Pd–C under an atmosphere of hydrogen. The solution was filtered and the solvents were removed under reduced pressure. The residue was sublimed to provide material, m.p. 243–245°, unchanged by crystallization from ethyl acetate; $[\alpha]^{24}D$ −11°, $[\alpha]^{24}_{436}$ −22° (*c* 0.68, ethanol).

Acknowledgment.—We wish to thank Professor K. Biemann in whose laboratory preliminary mass spectrometric studies were carried out by A. L. B.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO., KALAMAZOO, MICHIGAN]

Celesticetin. III. The Partial Structure[1-3]

BY HERMAN HOEKSEMA AND JACK W. HINMAN

RECEIVED JUNE 23, 1964

The reactions and determinations leading to the partial structure of celesticetin are presented.

Celesticetin is an antibiotic which has activity against Gram-positive organisms both *in vitro* and *in vivo*.[1] It is readily extracted from fermentation broths by organic solvents under neutral conditions.[2] The compound is most conveniently isolated as the crystalline oxalate or salicylate salts. These are reconverted with ease to an amorphous free base which has been shown to be 96% pure by countercurrent distribution. The compound has the molecular formula[4] $C_{24}H_{36}N_2O_9S$ and contains one C-methyl, one N-methyl, and one methoxyl group. Potentiometric titrations in water and dimethylformamide reveal a basic function of pK_a' 7.7, and an acidic one of pK_a' 9.8, with an equivalent weight per group of 517 ± 20.

Structural studies show celesticetin to have the partial structure **1**. This is established by a study of celesticetin derivatives, the products of acidic and basic hydrolyses, and nickel desulfurization.

Celesticetin base displays characteristic salicylate ester type absorption in the ultraviolet with maxima in 0.01 *N* alcoholic potassium hydroxide at 248 mμ, $E^{1\%}_{1\text{ cm}}$ 130.3, and at 341 mμ, $E^{1\%}_{1\text{ cm}}$ 103.7. In 0.01 *N* ethanolic sulfuric acid the maxima shift to 240 mμ, $E^{1\%}_{1\text{ cm}}$ 183.7, and to 310 mμ, $E^{1\%}_{1\text{ cm}}$ 80.6. In chloroform solution, infrared absorptions at 3300 cm.$^{-1}$ characteristic of hydroxyl and imino functions, at 1670 cm.$^{-1}$ originating, as will appear below, in a salicylic ester grouping, and at 1645 and 1517 cm.$^{-1}$ suggesting a monosubstituted amide, can be distinguished. Positive ferric chloride and Molisch tests, and negative Benedict, ninhydrin, and iodoform reactions are observed. Precipitates may be obtained with bromine water, Millon's reagent, and mercuric chloride. In aqueous solution, celesticetin consumes 4 moles of periodate rapidly, and part of an additional one slowly. Of these, two would be expected to oxidize sulfur to the sulfone.

Acetylation of celesticetin affords a tetraacetate, **2**, $C_{32}H_{44}N_2O_{13}S\cdot HCl$, crystalline as the hydrochloride. The only titrable group remaining in **2** is a basic function, pK_a' 7.7.

The presence of a salicylate ester is confirmed chemically by alkaline hydrolysis in 1 *N* sodium hydroxide. This provides an acid-insoluble fraction, identified as salicylic acid. The phenolic hydroxyl accounts for the weakly acidic function of celesticetin. From the filtrate, evaporated to dryness at pH 8.3, an alcohol-soluble material which fails to crystallize may be obtained. Countercurrent distribution in 1-butanol, water, and ammonia shows this amorphous substance to be 96% pure. This compound, desalicetin, **3**, retains the N-methyl, O-methyl, and C-methyl groups and has the molecular formula $C_{17}H_{32}N_2O_7S$. It is a base, pK_a' 7.8, which is optically active ($[\alpha]D$ +175° (*c* 1, ethanol)) and shows only end absorption in the ultraviolet. The ester and aromatic bands in the infrared have disappeared, but those attributed to the monosubstituted amide, now shifted slightly to 1650 and 1525 cm.$^{-1}$, remain. Desalicetin is cleaved by 4 moles of periodate rapidly and part of an additional mole slowly. Following removal of excess periodate and acidic hydrolysis, treatment with 2,4-dinitrophenylhydrazine permits isolation of glyoxal 2,4-dinitrophenylosazone. Desalicetin also gives a tetraacetate upon acylation with acetic anhydride. This can be crystallized as the hydrochloride, $C_{25}H_{40}N_2O_{11}S\cdot HCl$ (**4**). Although desalicetin has low *in vitro* antibacterial activity, it is interesting to note that it is fully as active *in vivo* on a molecular basis as is celesticetin.

Desulfurization of celesticetin with Raney nickel, which has been washed with a citric acid buffer at pH 3 to minimize alkaline hydrolysis, affords ethyl salicylate as an ether-soluble fragment. This shows that the salicylic acid moiety is bound through an ester linkage to a two-carbon fragment. One of these two carbons must then be linked to the sulfur atom.

The water-soluble product of desulfurization is isolated by evaporation at pH 8, and extraction of the residue with ethanol, then conversion to the hydrochloride, $C_{15}H_{28}N_2O_6\cdot HCl$. This is called 1,5-anhydrocelesticetitol, **5**. This optically active, nonreducing material can also be obtained by desulfurization of desalicetin. It has one basic function, pK_a' 7.8. Only 2 moles of periodate are consumed rapidly by **5**, again with slow additional uptake; 1 mole of formic acid is liberated. The desulfurized product contains three acylable hydroxyls, which can be acetylated to form a crystalline triacetate hydrochloride, $C_{21}H_{34}N_2O_9\cdot HCl$, **6**. In view of the periodate data these hydroxyls must be adjacent. Since the fourth oxygen in **5** is in the methoxyl function, and the fifth in the

(1) C. DeBoer, A. Dietz, J. R. Wilkins, C. N. Lewis, and G. M. Savage, "Antibiotics Annual (1954–5)," Medical Encyclopedia, New York, N. Y., p. 831.

(2) H. Hoeksema, G. F. Crum, W. H. Devries, *ibid.*, (1954–5), p. 837.

(3) J. W. Hinman and H. Hoeksema, presented in part at the 126th National Meeting of the American Chemical Society, Dallas, Texas, April, 1956.

(4) The analytical data actually require a range of H_{36-40}.