[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF., AND THE FACULTAD DE QUIMICA Y FARMACIA, UNIVERSIDAD NACIONAL DE LA PLATA, LA PLATA, ARGENTINA]

The Structures of the *Aspidosperma* Alkaloids Spegazzinine and Spegazzinidine[1]

BY CARL DJERASSI, H. W. BREWER, H. BUDZIKIEWICZ, ORFEO O. ORAZI AND RENÉE A. CORRAL

RECEIVED APRIL 27, 1962

Spegazzinidine ($C_{21}H_{28}N_2O_4$), a new alkaloid from *Aspidosperma chakensis* Spegazzini, has been shown to possess structure III, thus making it the first naturally occurring dihydroindole alkaloid with a catechol system. Nuclear magnetic resonance spectrometry was largely responsible for elucidating the precise nature of the N-acetyldihydroindole fragment, while mass spectrometry demonstrated the existence of an aspidospermine-like skeleton. The nature of the remaining oxygen function was recognized as a hydroxyl group by oxidation to a ketone, which exhibited an unusual mass spectrometric fragmentation pattern. Chemical proof for structure III was provided by successive methylation of the catechol grouping, tosylation of the alcohol function and finally lithium aluminum hydride reduction (of VIII) leading to 16-methoxy-N-deacetyl-N-ethylaspidospermine (IX), which proved to be the antipode of the lithium aluminum hydride reduction product of (+)-pyrifolidine (antipode of V). In view of the known antipodal relationship of (+)-pyrifolidine (antipode of V) and (−)-aspidospermine (I), spegazzinidine can be defined as 3,16-dihydroxy-17-demethyl-(−)-aspidospermine, while mass spectrometric evidence coupled with n.m.r. measurements allow the assignment of 3-hydroxy-17-demethyl-(−)-aspidospermine (XII) to the related alkaloid spegazzinine.

The establishment of the structure of (−)-aspidospermine (I) by X-ray analysis[2] has made available a reference substance, the chemical and spectroscopic properties of which were used to great advantage during the past two years in the elucidation of the constitutions of a variety of alkaloids[3-12] with a related pentacyclic or hexacyclic skeleton. Two of the newer physical methods—n.m.r. and mass spectrometry—played a particularly important role in the rapid solution of these complicated structural problems and we should now like to report[13] on the use of these techniques in the structure determination of two additional members of this complicated class of alkaloids, namely spegazzinidine and spegazzinine.

Several years ago,[14] we reported the isolation of (−)-quebrachamine[15] together with a new alkaloid spegazzinine from the Argentinian *Aspidosperma* species *A. chakensis* Spegazzini. The analytical results did not differentiate firmly between the empirical formulas $C_{21}H_{28}N_2O_3$ and $C_{22}H_{30}N_2O_3$, but preference was expressed for the former because of a presumed biogenetic relationship to (−)-aspidospermine (I) and a knowledge of the functional groups present in the new alkaloid, which could be attributed to partial structure IIa. Insufficient material was available at that time to pursue further chemical studies. When new plant material was collected, it was observed that the earlier isolation procedure yielded only small amounts of spegazzinine, the principal alkaloid now being a more highly oxygenated one which we have named spegazzinidine. In view of its relative abundance, all further chemical studies were performed with it.

The elementary analysis of spegazzinidine (III), m.p. 237–238°, $[\alpha]_D$ + 186°, was consistent with the empirical formula $C_{21}H_{28}N_2O_4$ (372) and this was confirmed by the mass spectrum, which exhibited a strong molecular ion peak at *m/e* 372. The ultraviolet absorption spectrum (Fig. 1) was similar to that[14] of spegazzinine (XII) and suffered a bathochromic shift upon addition of alkali, indicative of the presence of a phenolic group, while the strong infrared band at 6.13 μ showed the existence of an amide function which would be consistent with an N-acyldihydroindole grouping so common among aspidospermine (I) and its relatives. The n.m.r. spectrum[16] proved to be most instructive and, as shown in Fig. 2, assignments could be made to a phenolic grouping, a hydrogen-bonded phenol, two *ortho* aromatic protons, an N-acetyl function and a C-ethyl group. The mass spectrum (see below) indicated the existence of the usual aspidospermine-type skeleton and since the n.m.r. spectrum[3a,3b,4] of aspidosper-

(1) This paper represents part XI in the Stanford series "Mass Spectrometry in Structural and Stereochemical Problems" (for preceding article see X. Monseur, R. Goutarel, J. Le Men, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Bull. Soc. Chim. France*, 1088 (1962)) and part VI in the La Plata series "Estudios Sobre Plantas" (for preceding article see T. Nakano, C. Djerassi, R. A. Corral and O. O. Orazi, *J. Org. Chem.*, **26**, 1184 (1961)).

(2) J. F. D. Mills and S. C. Nyburg, *J. Chem. Soc.*, 1458 (1960).

(3) Cylindrocarpine, cylindrocarpidine and pyrifolidine: (a) C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, J. N. Shoolery and L. F. Johnson, *Experientia*, **16**, 532 (1960); (b) C. Djerassi, B. Gilbert, J. N. Shoolery, L. F. Johnson and K. Biemann, *ibid.*, **17**, 162 (1961); (c) C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert and L. D. Antonaccio, *Tetrahedron*, **16**, 212 (1961).

(4) Aspidocarpine: S. McLean, K. Palmer and L. Marion, *Can. J. Chem.*, **38**, 1547 (1960).

(5) Aspidospermines differing in substitution patterns at positions 1, 2 and 17: K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters*, 485 (1961).

(6) Vindolinine: C. Djerassi, S. E. Flores, H. Budzikiewicz, J. M. Wilson, L. J. Durham, J. Le Men, M.-M. Janot, M. Plat, M. Gorman and N. Neuss, *Proc. Natl. Acad. Sci. U. S.*, **48**, 113 (1962).

(7) Pyrifoline and refractidine: B. Gilbert, J. M. Ferreira, R. J. Owellen, C. E. Swanholm, H. Budzikiewicz, L. J. Durham and C. Djerassi, *Tetrahedron Letters*, 59 (1962).

(8) Vincadifformine: C. Djerassi, H. Budzikiewicz, J. M. Wilson, J. Gosset, J. Le Men and M.-M. Janot, *ibid.*, 235 (1962).

(9) Refractine and aspidofractine: C. Djerassi, T. George, N. Finch, H. F. Lodish, H. Budzikiewicz and B. Gilbert, *J. Am. Chem. Soc.*, **84**, 1499 (1962).

(10) Pleiocarpine, pleiocarpinine and kopsinine: W. G. Kump, D. J. Le Count, A. R. Battersby and H. Schmid, *Helv. Chim. Acta*, **45**, 854 (1962).

(11) Vindoline: M. Gorman, N. Neuss and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).

(12) Tabersonine: M. Plat, J. Le Men, M.-M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa and C. Djerassi, *Tetrahedron Letters*, 271 (1962).

(13) For preliminary communication see C. Djerassi, H. W. Brewer, H. Budzikiewicz, O. O. Orazi and R. A. Corral, *Experientia*, **18**, 113 (1962).

(14) O. O. Orazi, R. A. Corral, J. S. E. Holker and C. Djerassi, *J. Org. Chem.*, **21**, 979 (1956); *Anales Asoc. Quim. Argentina*, **44**, 177 (1956).

(15) For structure see K. Biemann and G. Spiteller, *Tetrahedron Letters*, 299 (1961).

(16) For reasons outlined earlier (C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.*, **26**, 1192 (1961)) all signals are reported in p.p.m. as δ units (c.p.s./60) relative to tetramethylsilane (δ = 0).

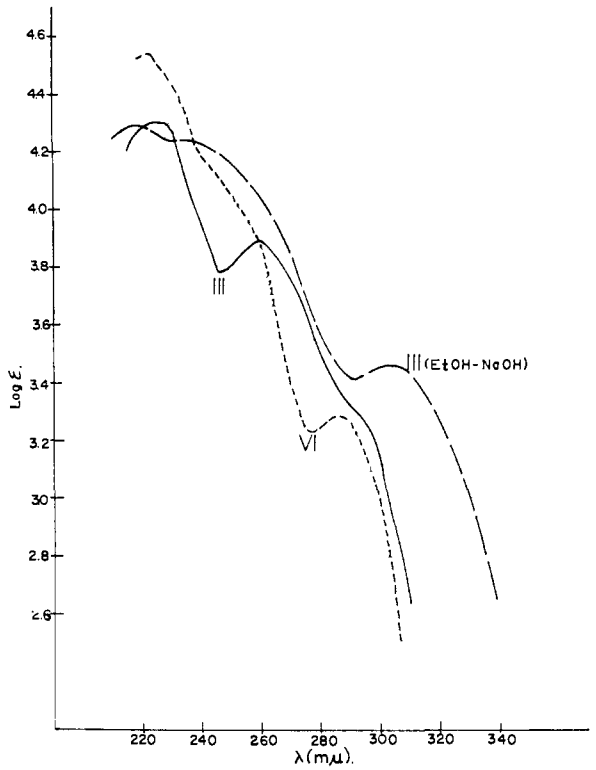


Fig. 1.—Ultraviolet absorption spectra of spegazzinidine (III) in ethanol and in ethanolic sodium hydroxide solution and of 3-dehydrospegazzinidine dimethyl ether (VI) in ethanol solution.

mine (I) showed a quartet in the 4.5 p.p.m. region due to the C-2 hydrogen with its *two* proton neighbors at C-3, the absence of such a signal in the spegazzinidine spectrum (Fig. 2) coupled with the presence of a doublet (J = 8 c.p.s.) at 4.05 p.p.m. suggested that the C-2 proton in spegazzinidine possessed only one neighboring hydrogen at C-3. The correctness of this assumption was established by the properties of the ketone VI described below.

Methylation of spegazzinidine (III) with dimethyl sulfate in acetone solution in the presence of potassium carbonate provided the 16,17-dimethyl ether IV, the n.m.r. spectrum of which was extremely similar to that[3b] of (+)-pyrifolidine (antipode of V[17]) except that the quartet of the latter in 4.5 p.p.m. region was replaced by a well-defined doublet centered at 4.32 p.p.m., the coupling constant (J = 8 c.p.s.) of which is typical[18] of coupling between two axial protons. These results define three of the four oxygen atoms of spegazzinidine in terms of the dihydroindole fragment contained in partial structures IIb or IIc and since spegazzinidine dimethyl ether (IV) still exhibits infrared hydroxyl absorption, the remaining oxygen function must be alcoholic in nature. Assuming the existence of an aspidospermine-type skeleton, this hydroxyl group would have to be located at C-3 (partial structures IIb or IIc) in order to explain

(17) No absolute configurations are implied in this article.

(18) See for instance L. M. Jackman "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 86.

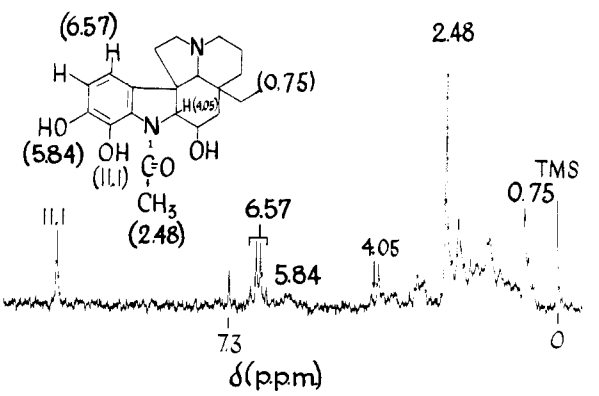


Fig. 2.—Nuclear magnetic resonance spectrum of spegazzinidine (III) in deuteriochloroform solution.

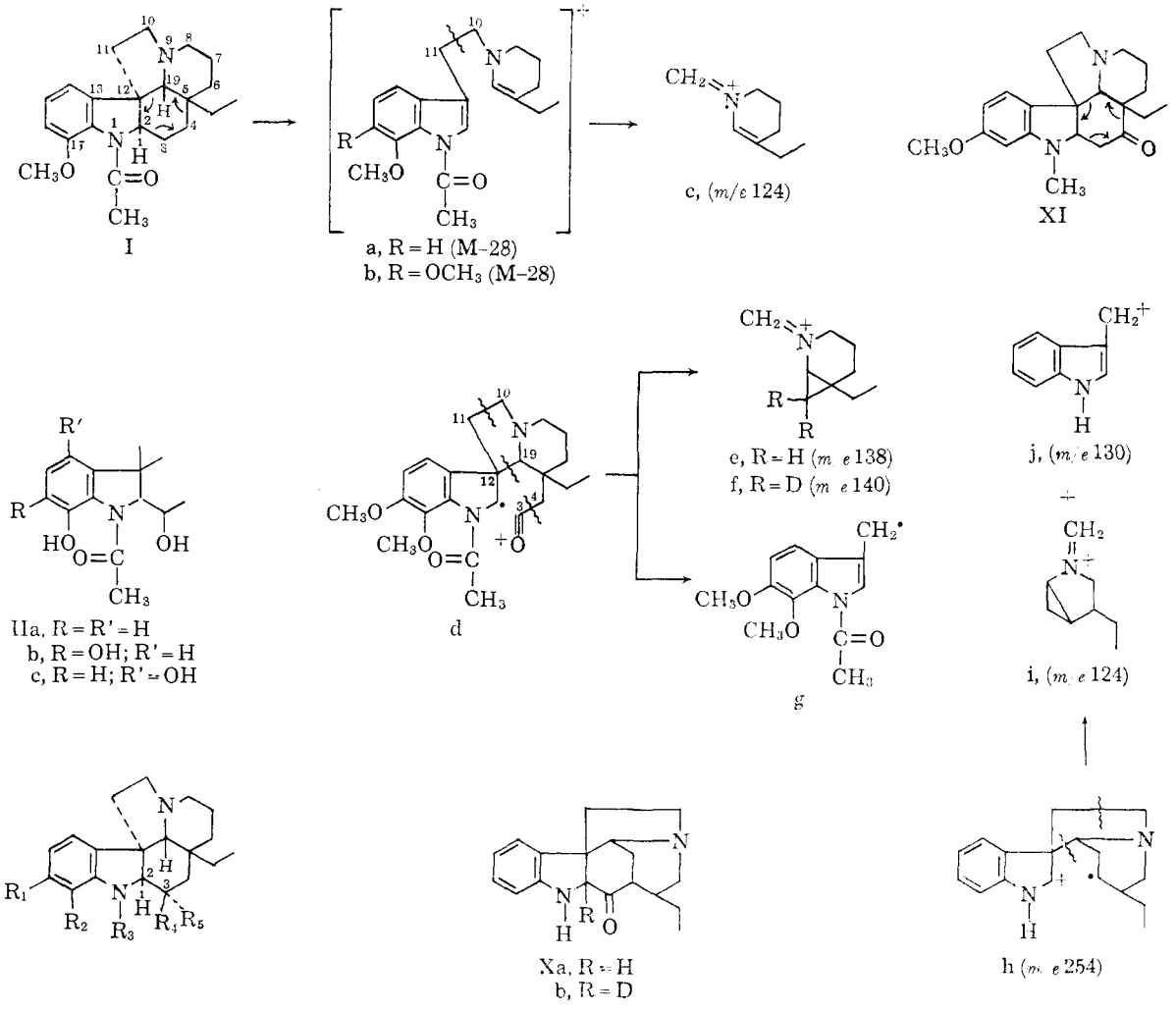
the doublet nature of the n.m.r. signal in the 4.2 p.p.m. region associated with the C-2 hydrogen. Chemical confirmation for these views was provided by oxidation of spegazzinidine dimethyl ether (IV) with chromium trioxide in sulfuric acid–acetone solution[19] to the ketone VI, the n.m.r. spectrum of which now showed a sharp singlet at 5.11 p.p.m., which can only be attributed to the C-2 hydrogen lacking any proton neighbors; the expected downfield chemical shift is caused by the adjacent carbonyl group.

Until this stage, the only basis for the assumption of an aspidospermine-like skeleton rests on biogenetic likeliness (co-occurrence with quebrachamine in *Aspidosperma* species and correspondence in empirical formulas after correcting for differences in functional groups) and the similarity of the n.m.r. spectra of spegazzinidine dimethyl ether (IV) and pyrifolidine (V), including the "aspidospermine fingerprint"[3a] region between 2.9–3.3 p.p.m. Strong support for this assumption could be provided by the following mass spectrometric measurements.

Biemann and collaborators[5] have shown that the mass spectra of aspidospermine (I) and its relatives show a peak at M-28, associated with the loss of ethylene (see arrows in I) and attributed to ion a, together with the most intense one at m/e 124 (ion c) produced by further fission of the 10–11 bond in a. Similarly, the strongest peak in the mass spectra of spegazzinidine (III) and spegazzinidine dimethyl ether (IV) (spectrum reproduced in ref. 13) occurs at m/e 124, thus strengthening greatly our assumption of an intact aspidospermine skeleton in spegazzinidine. The M-28 peak of aspidospermine[5] is not observed in the mass spectrum of spegazzinidine, but rather there is noted a M-44 peak, which can definitely be attributed to the loss of the two carbon bridge (C-3 and C-4) as the elements of $CH_2{=}CHOH$, since admixture of the dimethyl ether IV with heavy water prior to insertion into the mass spectrometer inlet system gave rise to an M-45 peak (loss of $CH_2{=}CHOD$).

The above n.m.r., chemical and mass spectral data are most consistent with structure III for spegazzinidine. Direct chemical verification for this working structure was provided by converting

(19) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).



	R_1	R_2	R_3	R_4	R_5
III	OH	OH	$COCH_3$	H	OH
IV	OCH_3	OCH_3	$COCH_3$	H	OH
V	OCH_3	OCH_3	$COCH_3$	H	H
VI	OCH_3	OCH_3	$COCH_3$	=O	
VII	OCH_3	OCH_3	$COCD_3$	=O	2,4,4-d_3
VIII	OCH_3	OCH_3	$COCH_3$	H	OTs
IX	OCH_3	OCH_3	C_2H_5	H	H
XII	H	OH	$COCH_3$	H	OH
XIII	H	OCH_3	$COCH_3$	H	OH

spegazzinidine dimethyl ether (IV) into its crystalline tosylate (VIII) followed by reduction with lithium aluminum hydride into the non-crystalline 16-methoxy-N-deacetyl-N-ethylaspidospermine (IX). Similar treatment of (+)-pyrifolidine (antipode of V)[3c] with lithium aluminum hydride provided N-deacetyl-N-ethylpyrifolidine (antipode of IX), which exhibited the same ultraviolet, infrared and mass spectra as well as thin-layer chromatographic mobility as IX derived from spegazzinidine, except that the two products exhibited rotations of opposite sign ($[\alpha]D$ $-20.6°$ *vs.* $[\alpha]D$ $+19.8°$). Since (+)-pyrifolidine has been shown[3a,3c] to be the antipode of O-methylaspidocarpine (V),[4] which has been related[4] chemically to (−)-aspidospermine (I), the latter and spegazzinidine (III) must possess the same absolute configuration. The relative configuration of (−)-aspidospermine has already been established[2,20] and the stereochemistry of the additional C-3 asymmetric center in spegazzinidine can be defined in terms of the stereoformula III (equatorial C-3 hydroxy group) because of the 8 c.p.s. coupling constant involving the axial protons at C-2 and C-3.

We have noted above that the appearance of an *m/e* 124 peak, first observed by Biemann[5] in the mass spectrum of aspidospermine (I), is indicative of an aspidospermine like skeleton. It should be pointed out however that this mass spectrometric feature should be evaluated with caution, since alkaloids with a different skeleton may show the same characteristic features, while a close relative of aspidospermine may at times give a very different mass spectrum. Both of these exceptions were encountered in the present study in carbonyl-containing alkaloids and while the mass spectral fragmentation patterns can now be rationalized in terms of the known structure III, consideration of the mass spectral data alone could have given rise to misinterpretation.[21] Thus, the mass spec-

(20) G. F. Smith and J. T. Wrobel, *J. Chem. Soc.*, 1463 (1960).

(21) It should be noted that this could be avoided in part by the use of a double focusing instrument, which could differentiate between the loss of ethylene (*m/e* 28.0403) and carbon monoxide (*m/e* 28.0038); see J. Beynon, "Mass Spectrometry and Its Applications to Organic Chemistry," Elsevier, Amsterdam, 1960.

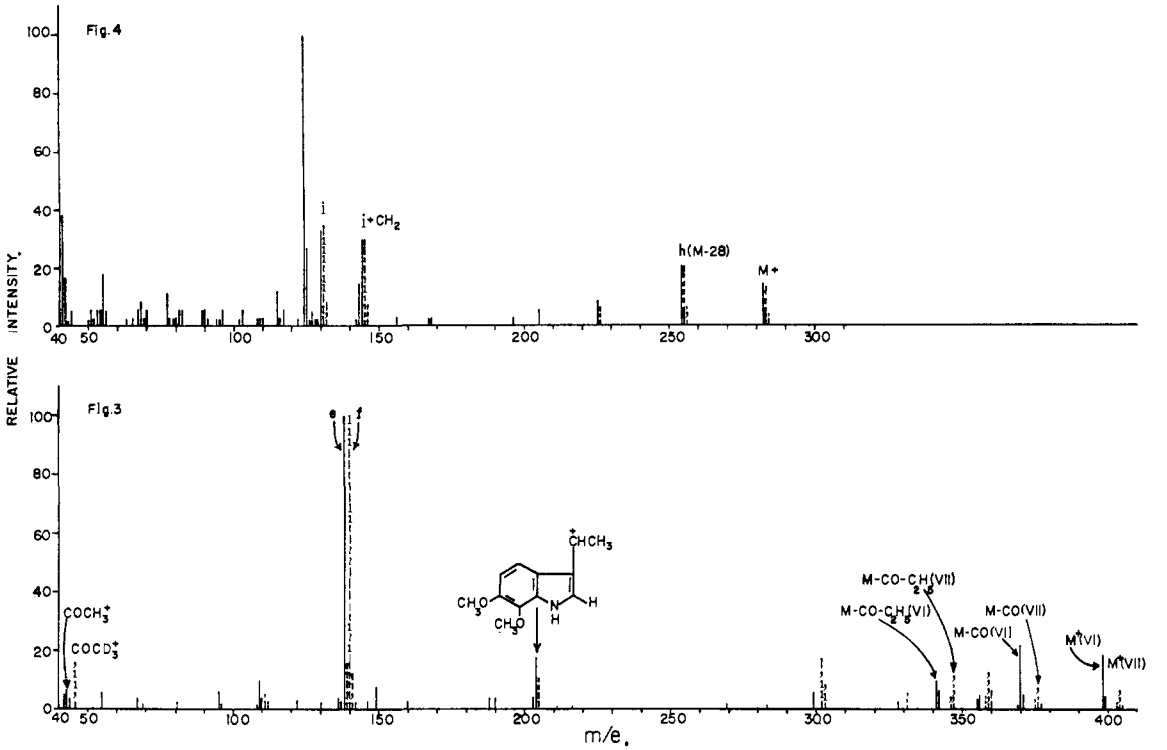


Fig. 3.—Mass spectrum of 3-dehydrospegazzinidine dimethyl ether (VI) and its hexadeuterio analog VII (only those peaks of VII, which are displaced with reference to those of VI are marked in broken lines.)

Fig. 4.—Mass spectrum of strychanone (Xa) and its monodeuterio analog Xb (only those peaks of Xb which are displaced with reference to those of Xa are marked in broken lines).

trum (Fig. 3) of the ketone 3-dehydrospegazzinidine dimethyl ether (VI) exhibits a M-28 peak (*m/e* 370) and no *m/e* 124 peak, but rather shows its most intense peak at *m/e* 138. Exhaustive deuteration of VI provided the hexadeuterio analog VII in which three deuterium atoms had entered adjacent to the carbonyl group, while the remaining three had replaced the acetyl hydrogens. This is demonstrated by the mass spectrum (broken lines in Fig. 3), where the molecular ion peak of VI was shifted from *m/e* 398 to 404 and the CH_3CO^+ peak at *m/e* 43 to 46 (CD_3CO^+), as well as by six unit shifts in the M-28 (loss of CO) and M-57 (loss of CO and C_2H_5) peaks. Most significant is the observation that the strongest peak at *m/e* 138 in the spectrum of VI is shifted by two mass units to *m/e* 140 in the spectrum of the hexadeuterio derivative VII. Consequently, an aspidospermine derivative with a carbonyl group at C-3 does *not* undergo the typical[5] aspidospermine mass spectral fragmentation (arrows in I *via* a to c) but rather suffers first cleavage of the 2–3 bond (typical of ketones[22]) to give ion d. Further fission of the 3–4 bond leads to expulsion of carbon monoxide generating the M-28 peak, while cleavage of the 3–4, 10–11 and 12–19 bonds in d would give rise to the stable radical g and the ion e (*m/e* 138). The latter still retains the two exchangeable hydrogens at C-5 and hence is found at *m/e* 140 (ion f) in the mass spectrum (shaded line in Fig. 3) of the deuterated ketone VII.

The recent degradation[23] of strychnine to strychanone (Xa) has made available another ketone in which the carbonyl group is located in the same position with respect to N_a as the spegazzinidine ketone VI and through the courtesy of Prof. Schmid of the University of Zurich, it has been possible to examine its mass spectrum (Fig. 4). Even though this substance does not belong to the aspidospermine family, its mass spectrum contains its most intense peak at *m/e* 124 and also shows a peak at M-28 (*m/e* 254). These results can be explained readily by assuming again initial expulsion of carbon monoxide with formation of h (*m/e* 254) followed by cleavage of the two bonds indicated by the wavy line (in h) with formation of i (*m/e* 124) and j (*m/e* 130). That this represents the correct sequence is shown by the recognition of metastable peaks at *m/e* 229.5 (*m/e* 282→254) and especially at *m/e* 61 (*m/e* 254→124), as well as by deuterium exchange. This treatment introduced only one deuterium atom (molecular ion at *m/e* 283), which must be located at C-2, since peaks h (*m/e* 254) and j (*m/e* 130) moved to *m/e* 255 and 131, while the principal peak at *m/e* 124 did not shift.

After completion of this work,[18] there appeared an article in which is reported[11] the degradation of the alkaloid vindoline to the ketone XI, which again follows the normal aspidospermine fragmen-

(22) See for instance pp. 354–360 in ref. 21 as well as F. W. McLafferty in "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1962, Vol. 2, Chapter 2, pp. 153–155.

(23) C. Weissman, O. Heshmat, K. Bernauer, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **43**, 1165 (1960).

tation path[5] (arrows in XI) with formation of ion c (*m/e* 124). It is also pertinent to mention that when a carbonyl group is located at C-6—as in certain degradation products of pyrifoline and refractidine—then the mass spectral fragmentation[7] proceeds in a "normal" fashion. In summary, caution should be exercised in the interpretation of mass spectra of such alkaloidal ketones and, whenever possible, peak assignments should be confirmed by deuteration experiments.

The complete structure elucidation of spegazzinidine (III) now permitted a rapid solution of the problem of the constitution of the earlier[14] isolated spegazzinine. The mass spectrum of the alkaloid exhibited a molecular ion at *m/e* 356, thus fixing the $C_{21}H_{28}N_2O_3$ empirical formula; the n.m.r. spectrum was extremely similar to that (Fig. 2) of spegazzinidine (III) except for the aromatic signals and the absence of a non-hydrogen bonded phenolic group (absence of the 5.84 p.p.m. signal). When this is combined with the earlier derived[14] partial formulation IIa, then structure XII follows almost automatically for spegazzinine. This conclusion was confirmed by comparing the mass spectra of speggazinidine (III) and spegazzinine (XII) as well as those of spegazzinidine dimethyl ether (IV) and spegazzinine methyl ether (XIII). Both pairs showed identical mass spectral fragments in the lower mass range including the most intense peak at *m/e* 124 (ion c). The first pair showed 16 mass unit differences in the higher mass range peaks, while 30 mass unit shifts were observed in the spectra (reproduced in ref. 13) of the methyl ethers. These shifts correspond in mass to the additional aromatic substituent and as has been noted by Biemann and collaborators,[5,15,24] and confirmed in our laboratory,[7,9] such an observation can be considered virtual proof of an identical carbon skeleton, the differences being restricted to the aromatic portion of the molecule.

The presence of a hydroxyl group at C-3—as has now been established for spegazzinidine (III) and spegazzinine (XII)—is so far unique among alkaloids related structurally to aspidospermine (I). Whether this is a biological "afterthought" or represents an important feature in the terminal stages of the biosynthesis[25] of these alkaloids remains an open question.

Experimental[26]

Separation of Spegazzinine and Spegazzinidine.[27]—A sample (5.0 g.) of crude oxalate (0.5% yield based on bark and isolated by ether precipitation as in ref. 14) was converted into the free base by partitioning between ether and 4% sodium bicarbonate solution: yield 3.8 g., m.p. 204–209°. This material represented a mixture of spegazzinine (XII) and spegazzinidine (III) as determined by paper chromatography (Whatman No. 1 paper pretreated with 20% formamide in acetone and dried for 45 seconds at 110°) using benzene saturated with formamide as the mobile phase and potassium iodoplatinate for detection: R_f 0.79 (spegazzinine) and R_f 0.45 (spegazzinidine). Crystallization from acetone–ligroin afforded 2.68 g. of alkaloid mixture (m.p. 230–232°) which was further purified by chromatography on 300 g. of Celite 545[28] which had been rolled overnight with 50 cc. of formamide and packed under nitrogen pressure into a column with benzene saturated with formamide. The alkaloid mixture was applied to the column in benzene solution and eluted with benzene saturated with formamide, 100-cc. fractions being collected. The benzene was removed from each fraction, the residue dissolved in chloroform, washed with bicarbonate solution, dried and evaporated. Fractions 5–10 (650 mg.) represented a mixture of spegazzinine (XII) and spegazzinidine (III), while fractions 11–18 (2.07 g.) contained pure spegazzinidine (III). The analytical specimen was obtained from acetone–isopropyl ether; m.p. 237–238°, $[\alpha]^{24}D$ +186°; $\lambda_{max}^{CHCl_3}$ 2.85, 6.13 and 6.33 μ; λ_{max}^{EtOH} 225, 260 mμ and 295 mμ (infl.); log ϵ 4.30 and 3.89; $\lambda_{max}^{EtOH-NaOH}$ 216, 237 and 304 mμ; log ϵ 4.29, 4.24 and 3.44 (Fig. 1); pK_a' 2.9, 6.4 and 10.7 in 33% dimethylformamide solution.

Anal. Calcd. for $C_{21}H_{28}N_2O_4$: C, 67.72; H, 7.59; N, 7.52; O, 17.18; mol. wt., 372. Found: C, 67.50; H, 7.64; N, 7.61; O, 17.29; OCH_3, 0.0; mol. wt., 372 (mass spec.).

Spegazzinidine Dimethyl Ether (IV).—To a refluxing mixture of 1.72 g. of spegazzinidine in 50 cc. of dry acetone, 2.0 g. of anhydrous potassium carbonate and 0.5 cc. of dimethyl sulfate were added 0.5-cc. portions of dimethyl sulfate at 12-hr. intervals. After 36 hr., water was added, most of the acetone removed under reduced pressure and the product isolated by extraction with methylene dichloride and chromatographed on 35 g. of neutral alumina (activity II). Elution with chloroform and recrystallization from acetone–isopropyl ether furnished 1.09 g. of the dimethyl ether, m.p. 167–169°, $[\alpha]^{25}D$ −156°, $\lambda_{max}^{CHCl_3}$ 2.96 and 6.20 μ; λ_{max}^{EtOH} 222, 250 and 290 (shoulder) mμ; log ϵ 4.48, 3.85 and 3.27; pK_a' 2.76, 6.45 in 33% dimethylformamide solution.

Anal. Calcd. for $C_{23}H_{32}N_2O_4$: C, 68.97; H, 8.05; OCH_3, 15.52; mol. wt., 400. Found: C, 69.02; H, 8.26; OCH_3, 15.27; mol. wt., 400 (mass spec.).

3-Dehydrospegazzinidine Dimethyl Ether (VI).—To a stirred solution of 153 mg. of spegazzinidine dimethyl ether (IV) in 20 cc. of acetone was added at room temperature dropwise over a period of 15 min. 0.4 cc. of 8 *N* chromium trioxide reagent,[19] a cloudy precipitate forming during the addition. The reaction mixture was left at room temperature for 10 min., isopropyl alcohol was added to decompose excess reagent, then water was added and most of the acetone was removed under reduced pressure. The residue was made alkaline with 10% sodium hydroxide, the product was extracted with methylene dichloride and chromatographed on 10 g. of neutral alumina, elution being effected with benzene–ether (4:1). Recrystallization from acetone–isopropyl ether gave 73 mg. of ketone, m.p. 155–160°, which was sublimed at 170° (10^{-6} mm.) for analysis; m.p. 185–187°, $[\alpha]D$ −53°, $\lambda_{max}^{CHCl_3}$ 5.84, 6.08 and 6.20 μ, λ_{max}^{EtOH} (Fig. 1) 222 and 285 mμ, log ϵ 4.54 and 3.28, pK_a' 3.0 and 5.48 in 33% dimethylformamide solution.

Anal. Calcd. for $C_{23}H_{30}N_2O_4$: C, 69.32; H, 7.59; mol. wt., 398. Found: C, 69.21; H, 7.52; mol. wt., 398 (mass spec.).

Deuterium exchange was performed by heating under reflux in an atmosphere of nitrogen 40 mg. of the ketone VI with 7 mg. of sodium, 1.5 cc. of deuteriomethanol and 0.5 cc. of heavy water. After 20 min., the solution was evaporated to dryness and this treatment was repeated twice more, whereupon 2 cc. of heavy water was added and the product extracted with ether. After drying over magnesium sulfate and evaporating to dryness, there was left 35

(24) K. Biemann, *Angew. Chem.*, **74**, 102 (1962).

(25) E. Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962).

(26) All melting points were determined on the Kofler block and all rotations were measured in chloroform solution. We are indebted to Dr. Lois J. Durham (Stanford University) and Dr. J. N. Shoolery (Varian Associates, Palo Alto, Calif.) for the n.m.r. measurements which were performed with Varian HR-60 or A-60 spectrometers in deuteriochloroform solution using tetramethylsilane as internal standard. The microanalyses are due to Mr. E. Meier and Mr. J. Consul of the Stanford University Microanalytical Laboratory, while the pK measurements were performed by Dr. H. Boaz of Eli Lilly Co., Indianapolis, Ind.

(27) This procedure was first worked out by Dr A. A. P. G. Archer at Stanford University.

(28) Commercial (John Manville and Co.) Celite 545 was allowed to stand overnight with 18% hydrochloric acid and the latter decanted. After washing to pH 5–6 with water, the portion of 100–200 mesh was washed to neutrality with water, then washed successively with acetone and methanol and dried overnight at 110°.

mg. of the hexadeuterio ketone VII, which was used directly for mass spectrometry (shaded lines in Fig. 3), the introduction of six deuterium atoms having been established by the molecular ion at *m/e* 404. A silica gel thin-layer chromatogram (acetone–ethyl acetate 1:4) demonstrated the presence of some deacetylated material, which accounts for the peaks at *m/e* 359 (2,4,4-trideuteriodeacetyl derivative), 331 (loss of CO) and 302 (loss of CO and C_2H_5).

Spegazzinidine Dimethyl Ether 3-Tosylate (VIII).—Freshly recrystallized *p*-toluenesulfonyl chloride (150 mg.) was added to an ice-cold solution of 175 mg. of spegazzinidine dimethyl ether (IV) in 5 cc. of dry pyridine and the resulting pink solution was left standing at room temperature overnight. Dilution with water and extraction with methylene chloride produced a purplish residue (233 mg.) which was chromatographed on 10 g. of neutral alumina (activity IV) and eluted with benzene. Recrystallization from methylene dichloride–isopropyl ether afforded 140 mg. of colorless crystals, m.p. 158–160°, $[\alpha]^{22}D$ +42°; $\lambda_{max}^{CHCl_3}$ 6.02, 6.25 and 8.5 μ.

Anal. Calcd. for $C_{30}H_{38}N_2O_6S$: C, 64.96; H, 6.91; S, 5.79. Found: C, 65.01; H, 6.85; S, 6.02.

Interrelation of Spegazzinidine and Pyrifolidine.—A solution of 143 mg. of pyrifolidine (antipode of V)[3c] in 10 cc. of ether was added dropwise to 0.5 g. of lithium aluminum hydride suspended in 20 cc. of the same solvent. After heating under reflux for 8 hr., excess reagent was decomposed by the dropwise addition of an aqueous saturated sodium sulfate solution and the product extracted with ether. The substance (antipode of IX) could not be crystallized and it was distilled at 115–120° (7×10^{-6} mm.), The infrared spectrum showed complete absence of the amide carbonyl band and thin-layer chromatography (1:1 acetone–ethyl acetate on silica gel using ceric sulfate reagent for spotting) revealed the presence of a single spot (R_f 0.72); λ_{max}^{EtOH} 218, 263 and 305 mμ; log ϵ 4.36, 3.80 and 3.48; $[\alpha]^{22}D$ +19.8°.

Anal. Calcd. for $C_{23}H_{34}N_2O_2$: C, 74.55; H, 9.25; mol. wt., 370. Found: C, 74.23; H, 9.16; mol. wt., 370 (mass spec.).

When the reduction of 172 mg. of the tosylate VIII was performed in the same manner, there was obtained 86 mg. of crude product, which exhibited two spots in a thin-layer chromatogram (R_f 0.72 and 0.51). The mixture was chromatographed on 10 g. of neutral alumina (activity IV) and the first fraction (32 mg.) eluted with benzene was distilled at 100–110° (3×10^{-6} mm.). The distillate represented 16-methoxy-N-deacetyl-N-ethylaspidospermine (IX) and proved to be identical in all respects (thin-layer chromatographic mobility, infrared and mass spectra) except for sign of rotation ($[\alpha]D$ −20.6°) with the above antipode derived from (+)-pyrifolidine.

Anal. Found: C, 74.51; H, 9.30; mol. wt., 370 (mass spec.).

2-Deuteriostrychanone (Xb).—Strychanone Xa (20 mg.) was heated under reflux in a nitrogen atmosphere with the same deuterium exchange mixture (NaOD–D_2O) described above. The procedure was repeated three times and the usual work-up afforded 15 mg. of crystalline residue, which was separated into two portions. One portion was used directly for mass spectrometry, while the other was warmed at 45° for 2 hr. with 10 cc. of 95% aqueous methanol, then taken to dryness and submitted for mass spectrometry. Both samples gave identical mass spectra with a molecular ion peak at *m/e* 283, indicating the incorporation of one deuterium atom.

Acknowledgment.—We are indebted to the National Institutes of Health of the U. S. Public Health Service for financial assistance (grants No. 2G-682 and A-4257) and to Prof. José F. Molfino for help with the botanical collection.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY, CAMBRIDGE 38, MASS.]

Studies on Synthetic Polypeptide Antigens. VI. The Synthesis and Physical Chemical Properties of a New Group of Linear-chain Antigens[1,2]

BY EDWARD FRIEDMAN, THOMAS J. GILL III[3] AND PAUL DOTY

RECEIVED MARCH 19, 1962

A group of synthetic polypeptides of immunological interest containing different combinations and proportions of L-glutamic acid, L-lysine, L-tyrosine, L-phenylalanine and L-alanine has been synthesized and the physical chemical properties determined. The polymers were in the molecular weight range of 30,000 to 550,000 and exhibited helical contents varying from 0–40% helix as determined by optical rotatory dispersion; all of the polymers were studied under physiological conditions of *p*H and salt. The intrinsic viscosities and sedimentation coefficients of the polymers showed a good correlation with the degrees of polymerization. Alanine residues were found to increase the amount and stability of the α-helical conformation in aqueous solution. One copolymer of L-glutamic acid, L-lysine and L-tyrosine in a mole ratio of 51:33:16 was found to associate in solution as a result of conversion to the β-form in a manner analogous to protein denaturation.

Introduction

Several of the preceding papers in this series[4–6] have described the immunological and physical chemical properties of a group of linear synthetic polypeptides. The present paper will describe the synthesis and physical chemical properties of a new group of polypeptide antigens of immunological interest.[7] Three of the new polypeptides contained alanine so that the effect of a residue with an aliphatic side chain on the physical chemical and antigenic properties could be studied. Copolymers composed mainly of L-lysine or L-glutamic acid and small amounts of either L-alanine or L-tyrosine were studied to determine whether or not small amounts of alanine or tyrosine could convert the non-antigenic poly-L-lysine and poly-L-glutamic acid into antigens.[7] The properties of additional copolymers of L-glutamic acid, L-lysine and either L-tyrosine or L-phenylalanine were also studied.[6]

The large variation in composition of these polymers also afforded the opportunity to investigate the effects of different residues on the helical stability of the polypeptides.

Experimental

Polymer Preparation.—The method of polymerization has been previously described in detail.[6] The polymers were prepared by the reaction of the appropriate mixtures of the

(1) Work supported by a grant from the National Science Foundation (G-7487).

(2) Preceding paper in this series; W. F. Anderson and T. J. Gill III, *Biochim. et Biophys. Acta*, **58**, 558 (1962).

(3) Junior Fellow of the Society of Fellows, Harvard University.

(4) T. J. Gill III and P. Doty, *J. Mol. Biol.*, **2**, 65 (1960).

(5) T. J. Gill III and P. Doty, *J. Biol. Chem.*, **236**, 2677 (1961).

(6) E. Friedman, T. J. Gill III, and P. Doty, *J. Am. Chem. Soc.*, **83**, 4050 (1961).

(7) The immunological properties will be described in an article by T. J. Gill III and P. Doty (in preparation).