Table I. Ratio of Protected Cyclic Pentapeptide and
Decapeptide after Cyclization of Various Linear
Pentapeptide Active Esters

| | Ratio of compd in product[a] | |
| | Cyclic mono- mer[b] | Cyclic dimer[c] |
p-Nitrophenyl ester of [l]		
$\overset{1}{H}$-$\overset{2}{Val}$-$\overset{3}{Orn}(\delta$-$Z)$-$\overset{4}{Leu}$-$\overset{5}{D}$-$\overset{}{Phe}$-Pro-OH	32	68
$\overset{5}{-Gly}$-OH[d,e]	79	21
$\overset{5}{-Sar}$-OH[f]	85	15
$\overset{4}{-Gly}$-Pro-OH[g]	25	75
$\overset{4}{-D}$-Ala-[h]	0	100
$\overset{4}{-D}$-Leu-[h]	+	++
$\overset{2}{-Lys}(\epsilon$-Z)- -D-Phe-[h]	29	71
$\overset{2}{-Dbu}(\gamma$-Z)-[h]	30	70
$\overset{1}{H}$-Gly-Orn(δ-Z)-[i]	100	0
$\overset{1}{H}$-Ala-[i]	91	9
$\overset{1}{H}$-Leu-[h]	78	22
$\overset{1}{H}$-$\overset{2}{Gly}$-Lys(ϵ-Z)-[h,i]	100	0
$\overset{1}{H}$-Gly-Orn(δ-Z)- $\overset{5}{-Gly}$-OH[k]	100	0
H- Orn(δ-Z)-Leu-D-Phe-Gly-Gly-OH[k]	100	0

[a] After cyclization of p-nitrophenyl ester with pyridine. The concentrations of linear pentapeptide p-nitrophenyl esters in pyridine were $\sim3 \times 10^{-3}$ M. [b] Mono-Z-substituted; the figures were derived by calculation on a molar basis. [c] Di-Z-substituted; the figures were derived by calculation in which 0.5 mole is used as a unit. [d] Isolation of cyclic decapeptide: see H. Aoyagi, et al., J. Am. Chem. Soc., 86, 5700 (1964); Bull. Chem. Soc. Japan, 38, 2138 (1965). [e] Isolation of cyclic pentapeptide: see H. Aoyagi, M. Kondo, T. Kato, S. Makisumi, and N. Izumiya, ibid., in press. [f] H. Aoyagi and N. Izumiya, ibid., 39, 1747 (1966). [g] R. Nagata, M. Waki, M. Kondo, H. Aoyagi, T. Kato, S. Makisumi, and N. Izumiya, ibid., in press. [h] N. Izumiya, et al., to be published. [i] M. Kondo and N. Izumiya, Bull. Chem. Soc. Japan, in press. [j] R. Schwyzer reported that the cyclization reaction of H-Gly-Lys(ϵ-Mz)-Leu-D-Phe-Pro-ONp with pyridine yields exclusively the dimer, cyclo-[Gly-Lys(ϵ-Mz)-Leu-D-Phe-Pro]₂; see Chem. Abstr., 57, 949 (1962). [k] M. Kondo, H. Aoyagi, T. Kato, and N. Izumiya, Bull. Chem. Soc. Japan, 39, 2234 (1966). [l] After the first compound listed, only variations of residue will be shown.

EtOH) (Anal. Calcd for $C_{30}H_{46}O_5N_6 \cdot HCl \cdot 4H_2O$: C, 53.04; H, 8.16; N, 12.37. Found: C, 52.73; H, 7.90; N, 11.97), R_f 0.93[9] and 0.85;[10] amino acid ratios in the acid hydrolysate, $Val_{1.0}Orn_{0.9}Leu_{1.0}Phe_{1.0}Pro_{1.0}$. Gramicidin S and cyclosemigramicidin S were distinguishable by paper electrophoresis (solvent, formic acid–acetic acid–methanol–water, 1:3:6:10, pH 1.8) and on a carboxymethylcellulose column (solvent, 0.2 M pyridinium acetate with 30% methanol).

The proportion of monomer VII in the crude product after cyclization is increased with decrease in the concentration of the pentapeptide active ester V in pyridine. Weight ratios of monomer VII and dimer VI in the crude product were found to be 29:71 at 30×10^{-3} M, 32:68 at 3×10^{-3} M, and 45:55 at 0.3×10^{-3} M V in pyridine. Table I shows the results obtained in this laboratory which are ratios of the protected cyclic monomer and the protected cyclic dimer in the crude products after the cyclization reaction of various linear pentapeptide active esters.

The antibacterial activity of cyclosemigramicidin S toward several microorganisms, E. coli, P. vulgaris, S.

aureus, B. subtilis, and M. avium, was examined. The cyclic pentapeptide had no retarding effect on the growth of any of the microorganisms, even at as high a concentration as 100 μg/ml of the assay medium. Synthetic or natural gramicidin S, however, showed substantial activity under the same conditions toward S. aureus and B. subtilis.

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Received January 3, 1967

Absolute Signs of Indirect Nuclear Spin–Spin Coupling Constants[1]

Sir:

There has been a large amount of recent interest in the absolute signs of indirect nuclear spin–spin coupling constants. One reason this interest developed was because of the apparent discrepancy between the experimental determination of the opposite relative signs of $J_{vic\text{-}HH}$ and $J_{gem\text{-}HH}$ in various ethane derivatives[2] and the theoretical prediction that both are positive.[3] It was subsequently proposed[4] that "absolute" signs of J may be found if it is assumed that J_{13CH} is positive for a proton directly bonded to carbon. In this way $J_{gem\text{-}HH}$ is shown to be negative.[5] Despite the fact that the positive sign of J_{13CH} rests on fairly firm theoretical ground,[6] it remains desirable to determine its absolute sign experimentally.[7] This communication describes such a determination.

If the molecules being studied could be partially oriented, a nonzero direct nuclear magnetic dipole–dipole interaction, D_{ij}, between nuclei i and j, would appear in the nmr spectrum and would either add to or subtract from the splitting due to J_{ij} depending upon the details of the orientation and the sign of J_{ij}. Such an experiment has been proposed[8] and attempted[9] for molecules with an electric dipole moment in an electrostatic field, but recent work[10] has shown that at the present time such orientational effects cannot be detected. An alternative method of producing oriented molecules involves the use of liquid-crystal solvents. It has been shown that nematic liquid crystals become oriented in strong magnetic fields, and, moreover, that molecules dissolved in the nematic liquid crystals are

(1) This research was supported by the National Institutes of Health and a Sun Oil Fellowship (B. J. L.).
(2) R. R. Fraser, R. U. Lemieux, and J. D. Stevens, J. Am. Chem. Soc., 83, 3901 (1961); F. Kaplan and J. D. Roberts, ibid., 83, 4666 (1961); F. A. L. Anet, ibid., 84, 1053 (1962); K. A. McLauchlan and D. H. Whiffen, Proc. Chem. Soc., 144 (1962); C. A. Reilly and J. D. Swalen, J. Chem. Phys., 35, 1522 (1961).
(3) M. Karplus and D. H. Anderson, ibid., 30, 6 (1959); H. S. Gutowsky, M. Karplus, and D. M. Grant, ibid., 31, 1278 (1959); M. Karplus, ibid., 30, 11 (1959).
(4) M. Karplus, J. Am. Chem. Soc., 84, 2458 (1962).
(5) F. A. L. Anet, ibid., 84, 3767 (1962).
(6) M. Karplus and D. M. Grant, Proc. Natl. Acad. Sci. U. S., 45, 1269 (1959); N. Muller and D. E. Pritchard, J. Chem. Phys., 31, 768, 1471 (1959); J. N. Shoolery, ibid., 31, 1427 (1959).
(7) R. A. Bernheim and B. J. Lavery, Bull. Am. Phys. Soc., [II] 11, 172 (1966).
(8) M. W. P. Strandberg, Phys. Rev., 127, 1162 (1962); A. D. Buckingham and E. G. Lovering, Trans. Faraday Soc., 58, 2077 (1962); A. M. Vasil'ev, Zh. Eksperim. i. Teor. Fiz., 43, 1526 (1962); Soviet Phys. JETP, 16, 1078 (1963).
(9) A. D. Buckingham and K. A. McLauchlan, Proc. Chem. Soc., 144 (1963).
(10) J. M. Deutch and J. S. Waugh, J. Chem. Phys., 43, 2568 (1965); 44, 4366 (1966); R. E. J. Sears and E. L. Hahn, ibid., 45, 2753 (1966).

also oriented.[11] The nmr spectra of the solute species displays direct dipole–dipole interactions, chemical-shift anisotropies, and nuclear electric quadrupole interactions (if the resonant nucleus possesses an electric quadrupole moment), in addition to the interactions observed in an isotropic liquid.

In the present experiment CH_3F was dissolved in p,p'-di-n-hexyloxyazoxybenzene under a pressure of approximately 10 to 20 atm. The proton nmr spectrum at 100.000 Mcps in the isotropic phase at 140° consists of a doublet with $|J_{HF}| = 45 \pm 1$ cps, while the proton spectrum in the nematic phase at lower temperatures consists of two sets of triplets. The splitting between individual components of a triplet arises from H–H direct dipole interaction while the splitting between the two triplets is due to H–F direct dipole–dipole interaction *plus* H–F indirect spin–spin coupling. The ^{19}F spectrum at 94.0764 Mcps in the isotropic phase at 140° consists of a quartet with $|J_{HF}| = 45 \pm 1$ cps, while in the nematic phase the quartet remains but is split by the H–F direct dipole–dipole interaction in addition to the indirect H–F coupling. In addition, both the ^{19}F and proton spectra are shifted downfield (externally referenced) in the nematic phase as compared with the isotropic phase. These shifts arise from the anisotropic nature of the magnetic shielding which is expressed as $\Delta\sigma_i = \sigma_{||} - \sigma_\perp$ for nucleus i where $||$ and $\perp$ refer to components of the magnetic shielding tensor parallel and perpendicular to the threefold molecular symmetry axis. The anisotropic motion responsible for the above features can be expressed[12] in terms of the probability per unit solid angle that the symmetry axis of CH_3F makes an angle θ with the applied magnetic field.

$$P(\theta) = \frac{1}{4\pi} + C_{3z^2 - r^2}\left(\frac{\sqrt{5}}{8\pi}\right)(3\cos^2\theta - 1) \qquad (1)$$

The factor $C_{3z^2 - r^2}$ is called a motional constant and takes values between $\sqrt{5}$ and $-\sqrt{5}/2$.

The determination of the absolute sign of one of the interactions, D_{ij}, J_{ij}, $\Delta\sigma_i$, and $C_{3z^2 - r^2}$, determines the absolute sign of all the others. Measurements of the ^{19}F resonance of CH_3F trapped in a single crystal of β-quinol clathrate at 1.3°K show that $\Delta\sigma_F$ is negative.[13] This result makes possible the assignment of the absolute signs of D_{HH}, D_{HF}, J_{HF}, $C_{3z^2 - r^2}$, and $\Delta\sigma_H$ in the following way. The average magnetic shielding, $\bar\sigma_{zz}$, of a nucleus in the semioriented molecule may be expressed[12] in terms of the isotropic magnetic shielding, $^1/_3(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$, and $\Delta\sigma$ as

$$\bar\sigma_{zz} = \frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz}) + C_{3z^2 - r^2}\left(\frac{2}{3\sqrt{5}}\right)\Delta\sigma \qquad (2)$$

with the z axis being identified with the molecular symmetry axis. Since σ_{zz} is less than $^1/_3(\sigma_{xx} + \sigma_{yy} + \sigma_{zz})$ for ^{19}F (downfield shift) in the nematic phase, and since $\Delta\sigma_F$ is negative, $C_{3z^2 - r^2}$ must be positive. This means that the molecule tends to be oriented with the symmetry axis (which happens to be the longest geometrical dimension) parallel to the applied magnetic field and long axis of the nematic liquid crystal.[14]

(11) A. Saupe and G. Englert, *Phys. Rev. Letters*, **11**, 462 (1963).
(12) L. C. Snyder, *J. Chem. Phys.*, **43**, 4041 (1965).
(13) E. Hunt and H. Meyer, *ibid.*, **41**, 353 (1964).
(14) The fluoromethanes CH_2F_2 and CHF_3 also orient with their

Table I. Summary of the Interactions Obtained from the Analysis of the Proton and ^{19}F Nmr Spectra of CH_3F in the Nematic Liquid Crystal p,p'-Di-n-hexyloxyazoxybenzene at 80°[a]

Inter-action	Split-tings, cps	D_{HH}	D_{HF}	J_{HF}	$C_{3z^2 - r^2}$
H–H	525	+350			+0.03910
H–F	234		−279	+45	+0.03873
H–F	234		−189	−45	+0.02623

[a] The positive value of J_{HF} results in the same motional constant as that obtained from the H–H interaction while a negative J_{HF} gives large disagreement.

A summary of the spectral analysis is given in Table I. It is seen that J_{HF} must be +45 cps in order that the motional constant obtained from the H–F interaction agree with that obtained from the H–H interaction.

The magnetic shielding anisotropy obtained for ^{19}F was $\Delta\sigma_F = -179 \pm 15$ ppm, which is about three times larger than the value of -66 ± 8 ppm obtained from the experiments on CH_3F trapped in a clathrate.[13] This discrepancy could be due to (a) incomplete alignment in the clathrate experiment or (b) a large difference in the effect of the matrix upon $\Delta\sigma$. We have observed a small solvent effect on $\Delta\sigma$ by repeating our measurement of the ^{19}F spectrum in p-n-hexyloxybenzoic acid where $\Delta\sigma_F = -211 \pm 12$ ppm. The magnetic shielding anisotropy for the protons in CH_3F is $\Delta\sigma_H = -28 \pm 4$ ppm, an unexpectedly large value.

From the knowledge of the relative signs of the coupling constants in $^{13}CHFCl_2$,[15] and if the absolute positive sign of $J_{gem\text{-}HF}$ determined in the present work holds for geminal H–F coupling in general, the absolute sign of $J_{^{13}CF}$ is found to be negative and the absolute sign of $J_{^{13}CH}$ is positive as predicted. The assignment of a positive sign to J_{HF} in CH_3F depends only upon the assumption that the sign of $\Delta\sigma_F$ is the same in p,p'-di-n-hexyloxyazoxybenzene solvent as it is in a β-quinol clathrate matrix.

The present results are also in agreement with a recent experiment[16] on $CHFCl_2$ in which the sign of J_{HF} was deduced from differences in the relaxation rates of the various magnetic resonance transitions.

longest geometrical dimension parallel to the long liquid-crystal axis: B. J. Lavery and R. A. Bernheim, to be published.
(15) G. V. J. Tiers, *J. Am. Chem. Soc.*, **84**, 3972 (1962).
(16) E. L. Mackor and C. MacLean, *J. Chem. Phys.*, **44**, 64 (1966).

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Biosynthesis of Gentiopicroside, a Novel Monoterpene

Sir:

The aglucone of gentiopicroside (**1**), a bitter principle glucoside,[1] possesses the same carbon skeleton, **6**, as the C_{10} moieties of *Yohimbé*, *Rauwolfia*, *Cinchona*, and *Strychnos* indole alkaloids. Moreover, within its chromophore is an enol ether grouping conjugated with a carboxyl, identical with that found in the cyclopentanoid monoterpenes or iridoids.[2]

(1) L. Canonica, F. Pelizzoni, P. Manitto, and G. Jommi, *Tetrahedron*, **16**, 192 (1961).
(2) L. H. Briggs, B. F. Cain, P. W. LeQuesne, and J. N. Shoolery, *Tetrahedron Letters*, **2**, 69 (1963).