silica gel. Trituration of the fraction enriched in the 17 β isomer with hexane gave 62 mg of pure substance **2**,[3] mp 133–134°.[9] The remaining fraction of tetracyclic material (30 mg) consisted of a mixture of 17α and 17β epimers. Both epimers could be used for the succeeding steps of the synthesis. The failure to detect any 11β-hydroxy tetracyclic product indicates a stereochemical behavior like that observed in the 11-methyl series.[1a] The stereoselectivity of the cyclization portends well for obtaining a single optically active cyclization product from enantiomerically pure substrate.[16]

The 17β epimer **2**[3] was acetylated (pure acetate, mp 108–110°[9]), then submitted to ozonolysis[5] to give the trione **12**,[3] followed by cyclodehydration.[5] The alkaline conditions of this last step effected saponification of the acetate and equilibration at C-17 giving, after chromatography on silica gel, an 84% yield[8,13] of racemic 11α-hydroxyprogesterone **13**[3] admixed with its 17α epimer (ratio ca. 76:24 by VPC). Crystallization from methanol gave the 17β form, mp 190–196°.[9] The NMR, solution ir, mass spectrum, and VPC (coinjection) behavior were identical with the corresponding properties of authentic (+)-11α-hydroxyprogesterone.

Thus a total synthesis of racemic 11α-hydroxyprogesterone has been achieved in ca. 15% overall yield in 16 steps from simple compounds. This represents approximately a 7% overall (22 steps) yield of ("racemic") hydrocortisone acetate.[4] Our synthesis may be compared with a well-known[17] total synthesis, which leads to optically active cortisone in "1% overall yield in 27 steps."[18]

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References and Notes

(1) For recent papers in this series see (a) W. S. Johnson and G. E. DuBois, *J. Am. Chem. Soc.*, preceding paper in this issue; (b) R. A. Volkmann, G. C. Andrews, and W. S. Johnson, *Ibid.*, **97**, 4777 (1975); (c) W. R. Bartlett and W. S. Johnson, *Bioorg. Chem.*, in press.
(2) In our first exploratory study (W. S. Johnson and T. A. Bryson, unpublished observations) involving attempts to cyclize the benzyl ether of 1, a trace of tetracyclic material seemed to be formed; however, follow-up experiments were not promising.
(3) This formula depicts only one enantiomer of a racemic pair.
(4) In a personal communication, Dr. Philip F. Beal, III, of the Upjohn Company has indicated that the commercial conversion of **13** into hydrocortisone acetate is accomplished in ca. 50% yield according to a simplified and refined version of the published method: J. A. Hogg, P. F. Beal, A. H. Nathan, F. H. Lincoln, W. P. Schneider, B. J. Magerlein, A. R. Hanze, and R. W. Jackson, *J. Am. Chem. Soc.*, 77, 4436 (1955). We assume that this conversion would work as well starting from the 17α epimer of **13**.
(5) W. S. Johnson, M. B. Gravestock, and B. E. McCarry, *J. Am. Chem. Soc.*, **93**, 4332 (1971).
(6) W. S. Johnson, T-t. Li, C. A. Harbert, W. R. Bartlett, T. R. Herrin, B. S. Staskun, and D. H. Rich, *J. Am. Chem. Soc.*, **92**, 4461 (1970).
(7) Evaporative bulb-to-bulb distillation using a Buchi Kugelrohrofen.
(8) From an experiment performed by A. J. Lewis.
(9) (a) The NMR and ir spectra were entirely consistent with the assigned structure. (b) Satisfactory C, H analyses were obtained.
(10) Procedure developed by R. S. Brinkmeyer. The first specimen of the thioacetal **5** was prepared by T. A. Bryson from pure primary chloride.
(11) Cf. R. L. Markezich, W. E. Willy, B. E. McCarry, and W. S. Johnson, *J. Am. Chem. Soc.*, **95**, 4414 (1973).
(12) Procedure developed by M. Hendrick. The first specimen of **8** was prepared by B. E. McCarry.
(13) From an experiment performed by J. Calzada.
(14) For the stereospecificity of reduction of propargylic alcohols, see B. Grant and C. Djerassi, *J. Org. Chem.*, **39**, 968 (1974), and references cited therein.
(15) This yield has not yet been optimized. R. S. Brinkmeyer has recently found conditions (50% TFA in TFE, 6 h, 25°) which gave the tetracyclic product in 39% yield.
(16) See ref 1a for a discussion of this problem. In preliminary experiments by R. S. Brinkmeyer, optically active 1, of unknown enantiomeric composition, was obtained by asymmetrically induced hydride reduction of the ketone prepared by Jones oxidation of 1.[3] This sample of 1 did indeed give optically active **2**; hence the chiral center at pro-C-11 is not racemized completely (if at all) during cyclization. Work in progress is aimed at obtaining optically active intermediates efficiently at an early stage of a revised synthesis of 1.
(17) L. Velluz, G. Nomine, and J. Mathieu, *Angew. Chem.*, **72**, 725 (1960); L. Velluz, G. Nomine, J. Mathieu, E. Toromanoff, D. Bertin, R. Bucourt, and J. Tessler, *C. R. Acad. Sci.*, **250**, 1293 (1960).
(18) K. Nakanishi, T. Goto, S. Ito, S. Natori, and S. Nozoe, "Natural Products Chemistry", Vol. 1, Academic Press, New York, N.Y., 1974, pp 499–500.

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Determination of Signs of Long-Range Carbon–Carbon Coupling Constants. The SPT-Difference Spectroscopy Method

Sir:

In a recent investigation on correlations between long-range ^{13}C–^{13}C, ^{13}C–^{1}H, and ^{1}H–^{1}H NMR spin–spin couplings in geometrically equivalent systems, Marshall et al.[1] synthesized a tri-^{13}C-labeled methyl tetrolate (15-step scheme) in order to determine the signs of the ^{13}C–^{13}C coupling constants involving the labeled sites using double or triple resonance techniques. Although the preparation of multiply ^{13}C-labeled compounds is both tedious and expensive, it was indicated that such multiple labeling work is being extended to other compounds for determination of signs of J_{CC} and J_{CC}/J_{CH}. We wish to demonstrate that the signs of ^{13}C–^{13}C couplings may often be obtained most conveniently from *mono*-^{13}C-labeled compounds[2] using various ^{13}C–{^{1}H} double resonance techniques. For small molecules, such as methyl [1-^{13}C]tetrolate (**1**), with first-order ^{1}H and ^{13}C spectra both (1) the selective population transfer (SPT) method[3] and (2) the off-resonance (selective) proton decoupling technique[4] are useful whereas for second-order systems method 2 is in general preferable.[5]

1

The methyl tetrolate **1**, enriched with >90% ^{13}C at position C1 only and synthesized in three steps from propyne and >90% ^{13}C-enriched barium carbonate, serves to illustrate the determination of signs of ^{13}C–^{13}C couplings from monolabeled compounds. Magnitudes of the ^{13}C–^{13}C and ^{13}C–^{1}H couplings were obtained from first-order analysis of the proton decoupled and/or coupled ^{13}C spectra.[6]

The signs of $^{1}J_{C1-C2}$, $^{2}J_{C1-C3}$, and $^{3}J_{C1-C4}$ (127.5, 20.28, and 1.90 Hz, respectively) were all determined relative to that of $^{4}J_{C1-H5}$ using ^{13}C–{^{1}H} SPT experiments;[3] i.e., selective SPT π pulses were applied to transitions in the H5 proton region (corresponding to definite spin states for carbon C1) prior to observing the C2, C3, and C4 spectra, respectively. For all three cases J_{C1-CX} (X = 2, 3, and 4) and $^{4}J_{C1-H5}$ were found to be of opposite sign. The determination of $^{2}J_{C3-C1} \times {}^{4}J_{C1-H5} < 0$ from the C3 spectrum (doublets of quartets, $|^{2}J_{C3-C1}|$ = 20.28 Hz and $|^{2}J_{C3-H5}|$ = 10.74 Hz) is illustrated in Figure 1. Application of selective π pulses to the high frequency transition in the $^{13}C3$–H5 proton satellite spectrum (doublet of doublets, $|^{2}J_{C3-H5}|$ = 10.74 Hz and $|^{4}J_{C1-H5}|$ = 1.96 Hz) gives rise to emission and enhanced absorption within the low frequency quartet of the C3 spectrum (Figure 1b). The same effects are observed in the high frequency quartet when the gated perturbation is applied at 2.0 Hz *lower* frequency (Figure 1c). Thus $^{2}J_{C3-C1} \times {}^{4}J_{C1-H5} < 0$.

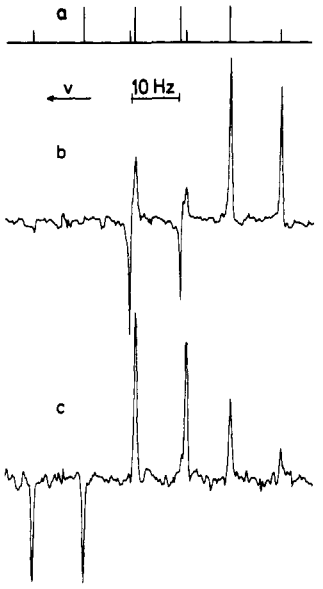

Figure 1. ^{13}C FT SPT NMR spectra of carbon C3 in **1** illustrating the determination of a negative sign for $^2J_{C3-C1} \times {}^4J_{C1-H5}$ (see text). (a) Stick plot showing the normal coupled C3 spectrum as a doublet of quartets ($|^2J_{C3-C1}| = 20.28$ Hz and $|^2J_{C3-H5}| = 10.74$ Hz). In (b) and (c) selective π pulses have been applied at the positions +6.35 Hz (b) and +4.35 Hz (c) to higher frequency from the methyl proton (H5) chemical shift, i.e., at the positions of the high-frequency doublet, corresponding to two different spin states for C1, in the H5 spectrum (doublet of doublets, $|^2J_{C3-H5}| = 10.74$ Hz and $|^4J_{C1-H5}| = 1.96$ Hz). Besides the −11:−9:15:13 pattern expected theoretically[3b] for only one of the quartets in each experiment, the spectra (b) and (c) show minor SPT effects for the other quartet due to partial perturbation of the degenerate H5 transitions corresponding to opposite C1 spin state. For both spectra $\gamma H_2/2\pi = 0.2$ Hz; acquisition time, 4 s; pulse delay, 0 s; number of transients, 400.

Similarly, from $^{13}C6$-{$^1H7$} SPT experiments it was easily shown that $^2J_{C6-O-C1} \times {}^3J_{C1-H7} < 0$; neither the absolute nor the relative signs of these two coupling constants were considered in the experiments of Marshall et al.[1] Finally, all the above mentioned relative sign combinations were also obtained by method 2.

Experimental results and theoretical calculations have shown that $^1J_{C-C}$'s,[7,8] like $^1J_{C-H}$'s,[8,9] are positive in sign. Thus from the present results for **1** it may be concluded that $^4J_{C1-H5}$ is negative while both $^2J_{C1-C3}$ and $^3J_{C1-C4}$ are positive.

In order to obtain the absolute signs of $^2J_{C1-C6}$ and $^3J_{C1-H7}$ we investigated the possibility of determining the sign of $^2J_{C1-O-C6} \times {}^1J_{C6-H7}$ from observation of the C1-C6 satellites in the C1 carbon spectrum after SPT π pulses have been applied selectively to H7 proton transitions for definite spin states of carbon C6. However, the C1-C6 satellite spectrum, a doublet ($|^2J_{C1-O-C6}| = 2.28$ Hz) of multiplets (quartet of quartets, $|^3J_{C1-H7}| = 4.17$ Hz and $|^4J_{C1-H5}| = 1.96$ Hz), is hidden under the strong C1 signal from the enriched carbon in **1**, thus impeding its observation. This problem was overcome using the pulse sequence recently described for the observation of hidden lines by combination of the SPT method and difference spectroscopy.[10]

Figure 2 illustrates the C1-C6 satellite patterns observed in the C1 spectral region using the SPT-difference spectroscopy method and shows complete elimination of the strong, overlapping C1 signal from **1**. Gated irradiation of the highest frequency line in the C6-H7 proton satellites (doublet of doublets, $^1J_{C6-H7} = 147.7$ Hz and $|^3J_{C1-H7}| = 4.17$ Hz) gives rise to a difference spectrum (Figure 2a) showing only net SPT intensity enhancements for the low frequency C1

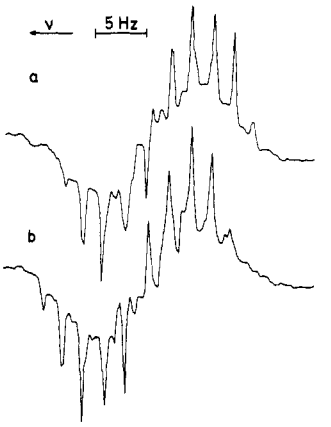

Figure 2. $^{13}C1$-{$^1H7$} SPT-difference spectra showing the C1-C6 satellite patterns obtained by application of selective π pulses at the C6-H7 proton satellite transitions: (a) gated irradiation at the transition at highest frequency; (b) gated irradiation at 147.7 Hz ($= {}^1J_{C6-H7}$) lower frequency. The spectra illustrate that $^2J_{C1-O-C6} \times {}^1J_{C6-H7} < 0$ (see text). For both experiments $\gamma H_2/2\pi = 0.8$ Hz; acquisition time, 4 s; number of (FID_π-FID_0) cycles,[10] 2000.

multiplet. Application of the SPT π pulse at 147.7 Hz ($^1J_{C6-H7}$) *lower* frequency results in a similar difference spectrum which, however, is shifted 2.3 Hz ($|^2J_{C1-O-C6}|$) to *higher* frequency (Figure 2b). This shows $^2J_{C1-O-C6} \times {}^1J_{C6-H7} < 0$ and thus it may be concluded that $^2J_{C1-O-C6} < 0$ and $^3J_{C1-H7} > 0$.

From similar $^{13}C1$-{$^1H5$} SPT-difference spectroscopy experiments, which involved the satellite spectra from $^{13}C4$, it was shown that $^3J_{C1-C4} \times {}^1J_{C4-H5} > 0$, thus confirming the above conclusions based on $^1J_{C1-C2} > 0$.

Our results agree with those of Marshall et al.[1] apart from minor deviations in the magnitudes for some of the couplings. Furthermore, we determined a negative sign for the small $^2J_{C1-O-C6}$ coupling and a positive sign for $^3J_{C1-H7}$. A negative sign has earlier been suggested for $^2J_{C-O-C}$ in dimethyl ether[11] and more recently this sign has been verified for $^2J_{C-O-C}$ in methyl chloroformate from ^1H-{^{13}C} Torrey oscillation experiments.[12]

The present experiments have shown that in many cases the signs of ^{13}C-^{13}C couplings may be most conveniently determined from mono-^{13}C-labeled compounds thus eliminating the need for multiple labeling.

References and Notes

(1) J. L. Marshall, D. E. Miiller, H. C. Dorn, and G. E. Maciel, *J. Am. Chem. Soc.*, **97**, 460 (1975).
(2) Recent studies indicate that in certain cases the signs of ^{13}C–^{13}C couplings may be determined even from nonenriched samples using the methods applied in this note.
(3) (a) S. Sorensen, R. S. Hansen, and H. J. Jakobsen, *J. Magn. Reson.*, **14**, 243 (1974); (b) H. J. Jakobsen, S. Aa. Linde, and S. Sorensen, *Ibid.*, **15**, 385 (1974); (c) A. A. Chalmers, K. G. R. Pachler, and P. L. Wessels, *Org. Magn. Reson.*, **6**, 445 (1974).
(4) (a) H. J. Jakobsen, T. Bundgaard, and R. S. Hansen, *Mol. Phys.*, **23**, 197 (1972); (b) S. Sorensen, R. S. Hansen, and H. J. Jakobsen, *J. Am. Chem. Soc.*, **95**, 5080 (1973).
(5) E.g., Applying the off-resonance proton decoupling method to the second-order spectra of methyl α-^{13}C-benzoate it was shown (unpublished results from this laboratory) that $^2J_{C2-C\alpha} \times {}^3J_{C\alpha-H2} > 0$ and $^3J_{C3-C\alpha} \times {}^4J_{C\alpha-H3} > 0$. Thus $^2J_{C2-C\alpha}$ ($= +2.49$ Hz) and $^3J_{C3-C\alpha}$ ($= +4.59$ Hz) are both positive since the ^{13}C–^1H couplings are known to be positive: A. M. Ihrig and J. L. Marshall, *J. Am. Chem. Soc.*, **94**, 3268 (1972).
(6) ^{13}C FT NMR spectra were obtained at 25.16 MHz on a Varian XL-100-15 spectrometer equipped with a gated Gyrocode decoupler. All SPT experiments were performed without allowing the system to relax fully between each pulse sequence; acquisition times of 4 or 8 s were used. Errors in coupling constants are $<\pm 0.1$ Hz. Experimental coupling constants determined for **1**, but not mentioned in the text, are: $^3J_{C2-H5} = 4.76$ Hz, $|^4J_{C2-H7}| = 0.56$ Hz, and $^1J_{C4-H5} = 133.0$ Hz.
(7) D. M. Grant, *J. Am. Chem. Soc.*, **89**, 2228 (1967).
(8) G. E. Maciel, J. W. McIvor, Jr., N. S. Ostlund, and J. A. Pople, *J. Am. Chem. Soc.*, **92**, 11 (1970).
(9) A. D. Buckingham and K. A. McLauchlan, *Proc. Chem. Soc., London*, 144 (1963).
(10) T. Bundgaard and H. J. Jakobsen, *J. Magn. Reson.*, **18**, 209 (1975).

(11) H. Dresskamp, K. Hildenbrand, and G. Pfisterer, *Mol. Phys.*, 17, 429 (1969).
(12) D. Ziessow, *J. Chem. Phys.*, **55**, 984 (1971).

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Table II. Intramolecularity of Hydrogen Transfer from the α-Carbon to C-3 Indole in the Tryptophanase-Catalyzed Decomposition of Tryptophan

Label in tryptophan	Solvent	% H/% D at C-3 of indole	% intramolecular transfer
α-H	D_2O	63.5/36.5	63.5
α-D	H_2O	92.1/7.9	7.9
α-D	D_2O	0/100	n.a.

Stereochemistry and Mechanism of Reactions Catalyzed by Tryptophanase and Tryptophan Synthetase

Sir:

The stereochemistry of some pyridoxal phosphate-catalyzed enzymatic β-replacement reactions of amino acids has recently been studied in several laboratories including this one. The synthesis of tryptophan from serine and indole or indole glycerol phosphate catalyzed by tryptophan synthetase (native or β_2 protein),[1,2] the formation of L-tyrosine from L-serine and phenol,[3] and the conversion of L-tyrosine to L-Dopa,[4] both catalyzed by tyrosine phenol-lyase, were all found to proceed with retention of configuration at C-3 of the amino acid side chain. We can now add to this list of examples another reaction, the synthesis of L-tryptophan from indole and L-serine catalyzed by tryptophanase. Using previously established methodology[1] and tryptophanase from *E. coli*, (2*S*,3*R*)- and (2*S*,3*S*)-serine-*U*-^{14}*C*-3-*t*[1] (T/^{14}C 7.9 and 5.3) gave samples of tryptophan (T/^{14}C 7.7 and 5.0), which were analyzed for their configurations at C-3 by feeding them to cultures of *Streptomyces griseus* ATCC 12648 to produce two samples of the antibiotic indolmycin of T/^{14}C 7.8 and 0.1, respectively. It had been previously established[1,5] that in the biosynthesis of indolmycin the *pro-R* hydrogen from C-3 of the tryptophan side chain is eliminated and the *pro-S* hydrogen is retained. Thus the above result shows that the tryptophanase-catalyzed synthesis of tryptophan from serine also occurs with retention of configuration at C-3.

We now wish to report on the steric course of the α,β-elimination reactions catalyzed by two enzymes, *E. coli* tryptophanase (Sigma Chemical Corp.) and tryptophan synthetase β_2 protein purified from *E. coli* mutant A2/F′A2. The deamination of L-serine to pyruvate and ammonia by either enzyme was examined using (2*S*,3*R*)- and (2*S*,3*S*)-serine-^{14}C-3-t and the deamination of L-tryptophan to indole, pyruvate, and ammonia by tryptophanase was studied with (2*S*,3*R*)- and (2*S*,3*S*)-tryptophan-*3*-^{14}*C*-*3*-*t*.[1] The reactions were carried out in D_2O, and the resulting pyruvate was trapped as lactate using an excess of lactate dehydrogenase and NADH. The lactate samples were isolated by paper chromatography (Whatman #3, 1-propanol:concentrated NH_4OH:H_2O 6:3:1) and oxidized to acetate.[6] The chirality of the methyl group in the acetate samples was then determined by the method of Cornforth et al.[7] and Arigoni et al.,[8] following Eggerer's procedure.[7] In this analysis procedure, which involves conversion to malate with malate synthetase followed by reaction with fumarase, (*R*)-acetate-*2*-d_1-*2*-t_1 gives rise to malate, which retains more than half of its tritium in the fumarase reaction, whereas the *S* isomer produces malate which retains less than half of its tritium in the fumarase reaction. The results of these experiments, which are summarized in Table I, show that in both deamination reactions catalyzed by tryptophanase the protonation at C-3 of the amino acid side chain occurs stereospecifically with retention of configuration. This finding parallels observations by Yang et al.[9] on the deamination of D-threonine by D-serine dehydratase and by Kapke[10] on the deamination of L-threonine by L-serine dehydratase, both of which also proceed with retention of configuration. In contrast, in the deamination of serine catalyzed by tryptophan synthetase β_2 protein, OH is replaced by hydrogen nonstereospecifically, suggesting as the most plausible explanation that the protonation at C-3 is in this case nonenzymic.[11] Although both types of enzymes operate through the same α-aminoacrylate-pyridoxal phosphate Schiff's base intermediate, these results point to a possible subtle difference between the enzymes primarily catalyzing α,β-eliminations and those primarily catalyzing β-replacement reactions: the presence in the former and absence in the latter of a base which can protonate C-3 of this common intermediate.

Finally, we determined the origin of the hydrogen at C-3 of the indole produced from tryptophan by tryptophanase. By carrying out the reaction with nonlabeled tryptophan in D_2O and with tryptophan-(*alanine-2-d*) in H_2O and D_2O and analyzing the indole for deuterium content at C-3 by FT-proton NMR it was established (Table II) that this hydrogen originated from C-2 of the amino acid side chain by a partially intramolecular transfer. These results suggest the following conclusions: (1) A single base on the enzyme catalyzes the proton abstraction from C-2 of the side chain, protonation of C-3 of the indole moiety, and, most likely, also protonation of C-3 of the aminoacrylate intermediate. (b) The proton transfer must be suprafacial. (c) The α,β-elimination of the indole is a syn elimination.

Table I. Stereochemistry of the Protonation at the β-Carbon Atom of Serine and Tryptophan in the Deamination Reaction Catalyzed by Tryptophanase or Tryptophan Synthetase β_2 Protein in D_2O

	Tryptophanase				Tryptophan synthetase β_2		Control	
	Serine-*3*-^{14}*C*-*3*-*t*		Tryptophan-*3*-^{14}*C*-*3*-*t*		Serine-*U*-^{14}*C*-*3*-*t*		Acetate-*2*-^{14}*C*-*2*-*d*, -*2*-*t*	
T/^{14}C of	3*R*	3*S*	3*R*	3*S*	3*R*	3*S*	2*R*	2*S*
Substrate	7.87	5.29	79.3	83.6	2.00	2.00	—	—
Lactate	8.11	5.73	76.0	79.8	2.09	2.04	—	—
Acetate	7.93	5.46	71.9[a]	74.3[a]	3.02	2.90	7.60	8.15
Malate	7.94	7.01	5.62	7.06	2.56	2.47	6.12	6.68
Fumarate	5.66	2.84	1.96	5.71	1.31	1.27	4.42	2.25
% T-retention of fumarase reaction	71.2	40.6	34.8	80.8	51.1	51.4	72.2	33.7

[a] T/^{14}C ratio readjusted by addition of ^{14}C reference compound.