Journal of Labelled Compounds — April-June — Vol. III, No. 2

A Semimicro Synthesis of Ferrocene (^{59}Fe) and some Derivatives useful as « Tags » for Proteins *

Lewis T. MANN, Jr.
Veterans Administration Hospital, West Roxbury, Mass. 02132
Received on 5th October 1966

SUMMARY

Ferrocene-^{59}Fe has been prepared with ferric chloride as the limiting reagent. Specific activities of five millicuries per millimole could be readily achieved. Ferrocene was converted to ferrocenylmethyl isothiocyanate and β-ferrocenylpropionic acid and these compounds were used in metabolic studies as "tags" for a series of synthetic polypeptides with molecular weights in the range of 50-100,000. Tagging at a level of about 5 microcuries per milligram of peptide obtained by direct reaction of the isothiocyanate with the ε-amino groups of lysine in a glutamic acid-lysine-tyrosine copolymer or by an activated coupling of ferrocenylpropionic acid and the synthetic polypeptide with a carbodiimide derivative. Some observations on the mechanism of tagging are included.

INTRODUCTION.

In order to study the potential utility of ferrocene derivatives as tracers of protein and other molecules in living animals, we have prepared ferrocene (^{59}Fe) and some of its derivatives on a semi-micro scale. Using modifications of some reported procedures [1, 2], we have investigated the preparation of ferrocene from aqueous ferric chloride, in which form iron-59 is commercially available. Because limitations in our laboratory facilities did not permit the use of more than five millicuries of iron-59 at any one time, we also directed our attention towards the use of iron as the limiting reagent. Isothiocyanate

* Work performed at the Department of Pathology, Harvard Medical School, Boston, Mass. 02115 and at the Veterans Administration Hospital, West Roxbury, Mass. 02132. Correspondence should be sent to the former address in the care of Dr. Thomas J. Gill, III.

derivatives of fluorescine and eosin are well-known as tags for protein molecules (antibodies), and we have studied similar derivatives of ferrocene (I). We chose ferrocenylmethyl isothiocyanate as the model tagging compound because of the well-documented instability towards air oxidation of ferrocene amine and its derivatives.

A mixture of carrier-low ferric (^{59}Fe) chloride, cold ferric chloride (as a carrier and redox indicator), and ordinary electrolytic iron powder (as the reductant) was stirred in N hydrochloric acid under nitrogen, until reduction was complete as indicated by fading of the yellow color. Ferrous chloride tetrahydrate ($FeCl_2 . 4H_2O$), obtained by evaporation of the solvent under nitrogen, was dehydrated to anhydrous ferrous chloride by heating to 160-180° C *in vacuo* (2 mm or less) [3]. This was converted to I in good yield by reaction either with cyclopentadienyl sodium in THF or with cyclopentadiene and diethylamine. The entire reaction was carried out without transfer of reactants until the final isolation step. The yield was very greatly depressed by traces of water and in event of an unsuccessful run, the residue from the reaction could be re-dissolved in *aqua regia*, and fumed down to ferric chloride. The iron might then be recycled by appropriate treatment, although with a consequent decrease in specific activity. Theoretically, activities of greater than two curies per millimole are attainable with this procedure.

Derivatives of I were prepared by micromodifications of reported procedures. I was converted to N, N-dimethylferrocenylmethylamine (II) methiodide [4]. Displacement of trimethylamine with sodio malonic ester afforded a solid derivative which was smoothly converted to β-ferrocenylpropionic acid (III) [5]. I was also converted to the bisulfite adduct of formylferrocene (IV) [6]. The oxime of IV was prepared, and the lower melting form [7, 8] was reduced with lithium aluminium hydride to the amine (V). Treatment of V with thiophosgene [9] yielded ferrocenylmethyl isothiocyanate (VI). The amine (V) was also prepared by the Gabriel synthesis [10] : II methiodide was heated with excess potassium phthalimide in DMF, to give the phthalimide of V which was easily cleaved with hydrazine hydrate to afford a good yield of V.

VI apparently may undergo spontaneous rearrangement to an equilibrium mixture of VI and the thiocyanate (VI A). The UV spectra of compounds prepared by the method of Nesmeyanov (II) or by the direct reaction of the amine (V) with thiophosgene are completely superimposable. However, while the IR spectra of VI and VI A are not identical, both show all of the same absorption bands, although in differing intensities. Therefore it appears that the stability of the ferrocenyl methyl cation is sufficiently great to permit reversal of the usual direction of rearrangement from thiocyanate to isothiocyanate.

II was also converted to ferrocenylacetonitrile (VII). Reduction of VII with lithium aluminium hydride afforded β-ferrocenylethyl amine (VIII) [12] an oil which decomposed slowly on standing. Attempts to isolate β-ferrocenyl-

ethyl isothiocyanate (IX) were not successful. Although VIII probably formed, conditions used in purification apparently caused a rearrangement to another product [13]. The ultraviolet and infrared spectra of VIII and its "rearrangement" product (IX A) showed significant differences (see Table I). Alkaline hydrolysis of VII yielded ferrocenylacetic acid (X) in good yield. However, X proved to be too unstable for use in protein tagging procedures. Perevalova *et al.* [14] have commented on the instability of this compound.

TABLE I. Comparison of Spectral Data of IX, before and after Sublimation.

Compound (Solvent)	Visible — UV		IR bands
	mμ max (ε)	mμ min (ε)	μ
IX — Before (isopropyl alcohol)	440 (100) (shoulders at 310-330 240-250)	362 (13.5)	4.75 [a] 8.45 12.0
IX — Before, ferricinium Salt (M $HClO_4$)	255 (1.93 × 10^4)	225 (8.4 × 10^3)	(not taken)
IX — After (isopropyl alcohol)	440 (100) 232 (72) 245 (6,500)	362 (13.5)	4.75 [a] 6.60 8.45 [b] 12.0 [b]
IX — After, ferricinium Salt (M $HClO_4$)	256 (1.29 × 10^4) shoulder at 281-287 (ca. 9.4 × 10^3)	227 (850)	(not taken)

[a] Intense.
[b] After ≫ before.

Tagging of synthetic polypeptides (SPP) has been accomplished by either of two procedures. First, reaction of VI with the ε-amino groups of the lysine residues of the SPP results in the formation of the thiourea derivatives or directly alkylated amino groups, presumably by displacement of the isothiocyanate group of VI. Analysis of tagged SPP's have indicated molar ratios as high as 4 : 1 or iron to sulfur, indicating that both mechanisms are operative. In addition recovery of the low molecular weight by-product, ferrocenylmethanol (XI), which was prepared independently by reduction of the aldehyde (IV) with lithium aluminium hydride, indicated that solvolytic displacement of the isothiocyanate (thiocyanate ?) group predominated over

hydrolysis. Second, and amide derivative can be formed by the reaction of an "activated complex" of a carboxylic acid derivative of ferrocene with the amine groups in the SPP. Either a carbodimide derivative [15] or an isoxazolium derivative [16] can be used as the "activating" agent. The use of tagged SPP's as model antigens is reported elsewhere [17].

EXPERIMENTAL *.

Ferrocene (^{59}Fe) : Carrier-low ferric chloride (^{59}Fe) (0.05-5.0 mc), as supplied in 1-2 ml of 0.5-1.0 N hydrochloric acid solution, was washed with 1-2 ml methanol (0.5 N in hydrochloric acid) into a 10 ml round bottom flask with a 14/20 joint. Cold ferric chloride ($FeCl_3 . 6 H_2O$, 180 mg, 0.67 mMole), and iron powder (electrolytic, 94%, 25 mg, ca 0.4 mMole) were added. The flask was closed with a straight condenser (100-150 mm long) with a nitrogen inlet and connection to a water aspirator. The contents of the flask were stirred under nitrogen at 40-50° C vigorously with a small "Teflon" coated magnet and reduction was essentially complete when the flask contents were decolorized (10-20 minutes). Solvent was removed under reduced pressure with care taken to avoid spattering (continuous stirring was advantageous). A greenish powder (contamined with black particles of unreacted iron), which remained after removal of the solvent, was further dried *in vacuo* (0.1-2.0 mm) at room temperature. Water of hydration was driven off by immersing the flask and contents *in vacuo* into a wax bath (160-180° C) for thirty minutes. The residue became a tan powder in about ten minutes. The anhydrous ferrous chloride was then cooled to 3-5° C, and dry nitrogen was cautiously admitted. Freshly depolymerized cyclopentadiene (0.35 ml, 3.8 mMoles), was added through the condenser with a long capillary dropper, followed by freshly dried and distilled diethylamine (1.0-1.5 ml, 0.71-1.07 g, 10-15 mMoles). Vigorous stirring was maintained under nitrogen as the reaction mixture was allowed to warm to room temperature, after 4-5 hours the solvent was removed under reduced pressure without heat.

The short condenser was replaced conveniently with an open cold finger trap-condenser, with vacuum connection. Ferrocene (and some dicyclopentadiene and diethylamine hydrochloride) sublimed onto the cold-finger (filled with ice and water) at 0.2-1 mm pressure and 70-90° C. ** The ferrocene was washed from the trap cold-finger with refluxing dichloromethane (2-3 ml). This solution after transfer to a small sublimator was evaporated

* Micro-analyses were performed by Professor Stephen Nagy, Dept. of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, and by Schwartzkopf Microanalytical Laboratory, Woodside, New York 11377.

All melting points were taken in micro-capillaries and are uncorrected. Boiling points are also uncorrected.

** Wherever vacuum was employed, all lines were double trapped with vacuum condensers in «Dry Ice»/acetone.

cautiously to dryness. I was sublimed readily at 0.5-1.0 mm pressure. The product melted at 175.0-175.5 (lit. val. 174-175° [2]) and did not depress with an authentic sample. Yields ranged from 94 mg to 166 mg (48-85%) based on 1.05 mMole total Fe. The average of four trials was 70%.

N, N-Dimethylaminomethylferrocene (II) Methiodide : II methiodide was prepared by the procedure of Lednicer and Hauser [4] with some minor modifications. A ten-fold molar excess of N,N,N′,N′-tetramethylmethylenediamine (bis-(dimethylamino)methane) was used; I was added in a few ml of ether solution; unreacted I was not removed prior to isolation of II; anhydrous magnesium sulfate was used in preference to sodium sulfate; 1 ml each of methanol and methyl iodide were used, and the volume of ether used for the final dilution was 20 ml. Starting with 300 mg (1.6 mMole) I, there was obtained 620 mg (1.6 mMole) of crude II methiodide (air dried).

β-ferrocenylpropionic acid (III) : into a small round bottom flask were placed freshly distilled dimethylsulfoxide (5 ml) and diethylmalonate (136 mg, 0.85 mMole). After a few minutes of gasing with dry nitrogen, sodium hydride (50% dispersion in mineral oil, 40 mg) was added quickly and allowed to react. Care was taken to exclude moisture. When the solid hydride had disappeared, II methiodide (270 mg, 0.7 mMole) was added. The mixture was stirred by bubbling nitrogen and heated to 190° C for 150 minutes (trimethylamine no longer detected with indicator paper). The product was worked up as described by Hauser and Lindsay [5], except that the final oil was not isolated but was transferred in ether solution to a small flask where the solvent was removed with a stream of nitrogen. Ethanol (1 ml), 45% potassium hydroxide solution (3 ml), and water (2 ml), were added, and the mixture was refluxed for 2 hours. After cooling, the mixture was diluted with 3 volumes of water and extracted once with ether. The aqueous layer was acidified carefully with concentrated hydrochloric acid (about 3.5 ml) and some ascorbic acid. The product was extracted twice with methylene chloride. After removal of solvent and drying at 100° C *in vacuo* (water aspirator) in a sublimator the product was heated to 140-160° C at 0.05-0.1 mm pressure. The product sublimed directly onto the cold finger. Yield : 109 mg (0.42 mMole, 60%), mp 107-115° C.

A "cold" preparation was recrystallized from *n*-heptane and sublimed *in vacuo* (0.005 mm) to yield canary yellow plates, mp 118.2-119.8°. (literature val : 116-118° [5]).

Bisulfite adduct of IV was prepared according to the procedure of Rosenblum *et al.* [7].

Oxime of IV : A solution of the bisulfite adduct of IV (prepared from 300 mg of I) in 2 N sodium hydroxide solution was extracted with methylene chloride. The methylene chloride layer was concentrated to 5 ml and stirred vigorously with solutions of hydroxylamine hydrochloride (1.5 g in 5 ml

water) and sodium carbonate (1.2 g in 5 ml water) overnight at room temperature. The organic layer and all solids were separated from the aqueous phase and evaporated to dryness with care to keep the temperature below 40° C.

The dark red residue was not isolated from the ^{59}Fe preparation. A parallel "cold" preparation afforded material melting at 105-108° C, with a yield of 80%. A higher melting isomer was recovered from the mother liquor, mp 125-130° C, raised by recrystallization to 137.5-140.5° C [18].

Ferrocenylmethylamine (V) : A solution of oxime of IV in dry THF was reduced with lithium aluminium hydride in THF, and the product recovered in the usual way.

The amine V isolated by extraction with methylene chloride was not purified from the ^{59}Fe preparation. In a parallel " cold" preparation, V from 4.0 g of oxime of IV was precipitated by shaking with cold 4 N hydrochloric acid 10 ml (containing 50-100 mg of ascorbic acid). The precipitate was filtered by suction, washed with methylene chloride, and dried overnight *in vacuo*. Yield : 3.4 g (78%). Recrystallized from ethanol-acetone, mp 170° (discolors) —180° (decomp), (lit. val. [19]; 185-190° darkens, crystal form to 250° C).

Anal : Calculated for $(C_{10}H_9)$ $FeCH_2NH_3$ Cl : C, 52.52; H, 5.61, Cl, 14.09; N, 5.57. Found : C, 52.01; H, 5.08; Cl, 14.28; N, 5.46.

V from II Methiodide : The procedure of Nesmeyanov *et al* [10] was adapted to micro quantities. From 156 mg of II methiodide and 300 mg of potassium phthalimide, V was prepared in 76% yield.

The "cold" amine (liberated by base and extracted with methylene chloride) was distilled through a short Vigreux column. Bp (0.2 mm) : 100-104°. Crystallizes readily, mp 31.5-33.0° (lit. val. : bp (0.3 mm); 108-110° [11]; mp : 30-35° C [19]). The free amine absorbs carbon dioxide very rapidly to form a light yellow solid.

Anal : Calculated for $(C_{10}H_9)$ $FeCH_2NH_2$: C, 61.42, H, 6.09; N, 6.51. Found : C, 61.15; H, 6.15; N, 5.99.

N-acetylferrocenylmethylamine : V (200 mg) was acetylated in a mixture of pyridine (1 ml) and acetic anhydride (0.4 ml). Recrystallized from acetone, yield : 175 mg (73%); mp, 145.5-147°; remelt : 145-147°.

Anal : Calculated for $(C_{10}H_9)$ $FeCH_2NHCOCH_3$: C, 60.72; H, 5.88; N, 5.45. Found : C, 60.90; H, 5.74; N, 5.52.

Ferrocenylmethyl isothiocyanate (VI) : To a solution of thiosphosgene (0.10 g, 0.95 mMole) in methylene chloride (8 ml) was added a suspension of V hydrochloride (100 mg, 0.5 mMole) in water (2 ml) containing 10-20 mg of ascorbic acid. To the mixture cooled in ice and stirred under nitrogen was added dropwise 0.1 N sodium hydroxide solution until the pH of the solution remained slightly basic (8-10 ml, 30-40 min). After stirring the mixture for 20 minutes more, the methylene chloride layer was separated from the aqueous

layer which was washed once with a small portion of methylene chloride. The combined organic layers were washed twice with 1 N hydrochloric acid (containing some ascorbic acid) and twice with saturated sodium chloride.

The organic layer was dried over magnesium sulfate, and the solvent evaporated to yield an orange-brown oil, which was sublimed *in vacuo* (0.05-0.1 mm, bath temperature no greater than 55° C) to yield light yellow crystals in less than maximum but satisfactory yield, as estimated by eye.

Parallel "cold" preparations afforded yields ranging from 36 to 60%. The cold material was conveniently purified by extraction of the crude oily product with petroleum ether (20-40°) or *n*-hexane. Glistening yellow plates, mp 56.5-58.0° C. Recrystallized from pet. ether, mp 63.5-64.5° C. The compound has a characteristic mustard-like odor.

Anal : Calculated for ($C_{10}H_9$) $FeCH_2NCS$: C, 56.05; H, 4.31; N, 5.45; S, 12.47. Found : C, 56.31; H, 4.00; N, 5.22; S, 12.61.

The ultraviolet spectrum in acetonitrile solution showed one clearly defined maximum at 438 mμ ($\varepsilon = 107$). A broad minimum was observed at 368 mμ. There are broad shoulders extending from 300-350 mμ and 230-290 mμ. The infra red spectrum showed a very strong absorbance band at 4.8 μ. ***

"*Ferrocenylmethyl thiocyanate*" *(VI A)* : Prepared according to Nesmeyanov *et al.* [19], this compound was obtained as a bright lemon yellow powder from petroleum ether (20-40), mp : sintered 46-47°, wet 53°, rapid melting 56.5-58.0° C. Recrystallized from petroleum ether (20-40) without change in melting point (lit. val. [11] : 59-61° C). On admixture of VI A with VI, the observed melting point was 56.0-59.0° C.

The ultraviolet spectrum in acetonitrile solution is identical with that of VI (above). The infra red spectrum showed a very strong band at 4.7-4.8 μ. Bands at 6.7, 6.9, 10, 11 and 12.5 μ differed qualitatively from VI, but were present in both spectra. The differences were more clearly observed in spectra taken in KBr pellet than in CCl_4 solution. ***

Ferrocenylacetonitrile (VII) : "Cold" II methiodide was converted to VII on a micro-scale by the method of Lednicer and Hauser [12]. Without significant modification, the yield was the same (70%).

β-ferrocenylethylamine (VIII) : "Cold" VII was reduced to VIII by the method of Lednicer and Hauser [12]. The yield was 94%. The brown oil decomposed slowly in storage (room temperature) over a 12-week period.

β-ferrocenylethyl isothiocyanate (IX) : "Cold" VIII (4.6 g, 0.02 Mole) was treated with thiophosgene, by the procedure of Kjaer, Marcus, and

*** Photocopies of the spectra will be furnished by the author on request.

Conti [9] as modified for the preparation of VI. Extraction of the crude product with petroleum ether (20/40) afforded orange-yellow leaflets, mp 45-50° with a characteristic thiamine-like odor. Yield : 3.5 g (65%) after sublimation *in vacuo* (0.05-0.1 mm, bath temperature 60-80° C); the product was almost odorless, mp 48.2 (wet); 48.6-49.6° C.

Anal : Calculated for $(C_{10}H_9)$ $FeCH_2CH_2NCS$: C, 57.57; H, 4.83; N, 5.17; S, 11.83. Found : C, 57.40; N, 4.65; N, 5.27; S, 12.08.

The UV and IR spectral data are compared in Table II.

TABLE II. Specific Activities of Ferrocene (Fe-59) Tagged SPP's.

SPP (mol. wt. average)	Tagged with	Specific activity of tag mC/mMole	Specific activity of tagged SPP	Iron Content (%)	Average number of tags per molecule of SPP
	VI	4.2	2.7 μC/mg	2.52	45
7A (70,000)	III	0.88	0.4 μC/mg	3.60	32
7ΔA (70,000)	VI	4.2	2.4 μC/mg	—	40
8C	VI (cold)	—		1.45	26
(100,000)	VI (cold)	—		0.44	8
	VI	4.2	0.59 μC/mg	—	14

Ferrocenylacetic acid (X) : "Cold" II (6.0 g) was hydrolyzed overnight in boiling 5 N potassium hydroxide in 50% ethanol (100 ml). The mixture was diluted with water and acidified with excess hydrochloric acid (and ascorbic acid), dissolved in ether, the ether layer was washed with saturated sodium chloride, dried over magnesium sulfate, and evaporated. Recrystallized from ethanol-water containing ascorbic acid; gold-yellow leaflets with a metallic sheen. Mp : 125-135° with *d.* (lit. val. [20] darkened 125-135, shrank and melted 135-140°). The dry ammonium salt decomposed at room temperature over a period of several weeks. The free acid was indefinitely stable.

Tagging of SBP 8c (X) ($Glu^{60}Lys^{40}$, MW = 100,000) with VI : SPP 8c (300 mg, about 820 Moles lysine) was dissolved in 60 ml of carbon dioxide free water. There was added 2 M sodium chloride (about 8 ml) and 0.5 M sodium carbonate/bicarbonate buffer (pH 9, 6-7 ml). The final pH was 9.15. To the cold solution, stirred in the cold (3-5° C) was added dropwise freshly

sublimed VI (75 mg, 0.29 mMole) in cold acetonitrile solution (7.5 ml) over a 12 hour period. After six hours further stirring in the cold, the solution was stirred for six hours at room temperature. A silky precipitate that had been present in the cold disappeared. The solution was briefly sedimented at about 1,000 g (max) for ten minutes and the clear supernatant solution decanted. The pH was lowered to 7.3-7.4 by dropwise addition of a solution of ascorbic acid (about 100 mg) in 1 N hydrochloric acid (10 ml). The volume of the solution (about 80 ml) was reduced to 13 ml by pressure dialysis. The dialysate was collected and reserved. Chromatography on Sephadex G-25 (coarse beads, exclusion volume 100 ml, eluant 0.2 M sodium chloride/0.01 M phosphate (pH 7.3) afforded a yellow band in about 60 ml of eluate after one exclusion volume. This was dialyzed 60 hours against two changes of a dilute buffer solution (0.01 M ammonium acetate) and then for 24 hours against distilled water. The dialyzed solution was freeze-dried and a pale yellow solid (238 mg) was recovered.

Anal : Content of SPP (biuret determination) : 99 + % for each of 2 typical products. Found for two typical products : (*a*) N, 12.31%; Fe, 1.45%; S, 0.21% or a molar Fe/S ratio = 3.9/1; (*b*) N, 12.07%; Fe, 0.44%; S, 0.098% or a molar Fe/S ratio of 2.6/1. The N content of untagged SPP 7*a* was 12.16% [21].

The dialysate (about 65 ml) was extracted with ether, which was dried over anhydrous potassium carbonate. The yellow-orange residue, remaining after evaporation of the ether to dryness, was recrystallized from hexane. The fine long needless thus obtained melted from 77-79° C.

Tagging of SPP 7a *(21) (Glu^{56}Lys^{38}Tyr^{6} MW = 70,000) with III* : SPP 7*a* (25 mg, about 64 micromoles of lysine) was dissolved in 5 ml of a pyridine/pyridine hydrochloride buffer (0.1 M in pyridine, pH 7.0). The buffer was carbon dioxide free, and the reaction was run under nitrogen. The mixture was stirred with a sealed stirrer. III (150 mg, 0.6 mMole) was added in 1 ml of solution, sufficient sodium hydroxide being added to bring III into solution at pH 7.0. A carbodiimide derivative (1-cyclohexyl-3-(2-morpholinyl-(4)-ethyl)-carbodiimide metho-*p*-toluene sulfate, abbreviated "CMC-methotosylate") was added as a dry powder (20 mg).

The pH rose to 7.3 and was adjusted to 7.0 with pyridine hydrochloride solution (1 M). An occasional addition of pyridine hydrochloride was required over the first hour period, during which time some turbidity appeared. After six hours, stirring was stopped, and the mixture was held at 0° C under nitrogen overnight. After 20 hours, a heavy precipitate had formed. The pH had again risen to 7.3, and the mixture had become a pea green.

Ascorbic acid (25-50 mg) was added to the solution, in order to reduce the ferricinium ion. The pH of the mixture was adjusted to 8 with N sodium hydroxide. The whole volume (about 10 ml) was chromatographed through Sephadex G-25 (coarse beads : exclusion volume 40 ml, eluant 0.2 M sodium

chloride, 0.05 M Tris/Tris Cl, pH 8). After a 37 ml fore-volume, a yellow band was collected in 19 ml. A second diffuse band appeared after another 20-30 ml and required about 300 ml for elution.

The first yellow band was reduced in volume by means of pressure dialysis to 4 ml and was dialyzed against several changes of distilled water for 48 hours. There was recovered 22 mg of SPP (biuret determination) containing an average tag density of 32 ferrocene tags per molecule of SPP 7*a* (containing 179 μMoles lysine). Ferrocene was determined as iron by wet ashing in a perchloric/nitric acid mixture; the ferric ion thus liberated was determined as the bathophenanthroline complex by a modification of the method of Diehl [22].

Ferrocenylmethanol (XI) : XI was prepared by reduction of the aldehyde (IV) with lithium aluminum hydride. Recrystallized from hexane, mp 74-76° C (lit. val. 74-76° C [23]). A mixed melting point of XI with the ether extracted material isolated from the dialysate of SPP tagging (above) melted at 74-77° C.

ACKNOWLEDGEMENT.

I should like to thank Dr. Wenzel Davidsohn, U.S. Army Natick Laboratories, for obtaining the IR spectra and to acknowledge the assistance of Miss Eila Kairinen in parts of this work. This work was supported in part by N.S.F. Grant GB-3603.

REFERENCES

1. WILKINSON, G. — *Org. Sym.*, **36** : 31-35 (1956).
2. EKEMARK, A. and SKAGIUS, K. — *Acta Chem. Scand.*, **16** : 1136-1138 (1962).
3. LINDSTROM, E. G. and BARUSCH, M. R. — U. S. Pat. 312557 (Cl 260-439), Feb. 25, 1964 to Cal. Res. Corp. (*Chem. Abs.*, **60** : 12057c (1964)). See also BLITZ and HUTTIG. — *Z. Anorg. Chem.*, **109** : 102 (1920).
4. LEDNICER, D. and HAUSER, C. R. — *Org. Syn.*, **40** : 31-33 (1962).
5. HAUSER, C. R. and LINDSAY, J. K. — *J. Org. Chem.*, **22** : 1246-1247 (1957).
6. ROSENBLUM, M. *et al.* — *J. Am. Chem. Soc.*, **85** : 316-324 (1963).
7. LINDSAY, J. K. and HAUSER, C. R. — *J. Org. Chem.*, **22** : 355-358 (1957).
8. PAUSON, P. *et al.* — *J. Chem. Soc.*, 650-656 (1950).
9. KJAER, A., MARCUS, F. and CONTI, J. — *Acta Chem. Scand.*, 7 : 1370-1374 (1953).
10. NESMEYANOV, A. N., PEREVALOVA, E. G., SHILOVTSEVA, L. S. and TYURIN, Yu. D. — *Izv. Akad., Nauk SSSR., Otdel. Khim. Nauk*, 1982 (1961); 1377 (1963).
11. NESMEYANOV, A. N., PEREVALOVA, E. G., SHILOVTSEVA, L. S. and USTINYUK, Yu. A. — *Doklady Akad., Nauk SSSR*, **124** : 133 (1959).
12. LEDNICER, D. and HAUSER, C. R. — *J. Org. Chem.*, **24** : 43-46 (1959).
13. ARIMOTO, F. S. and HAVEN, C. Jr. — *J. Am. Chem. Soc.*, **77** : 6295-6297 (1955).
14. PEREVALOVA, E. G., USTAYNYUK, Yu. A. and NESMEYANOV, A. N. — *Izv. Akad., Nauk SSSR, Ser. Khim.*, **11** : 1967 (1963).
15. SHEEHAN, J. C., CRUICKSHANK, P. A. and BASHART, G. L. — *J. Org. Chem.*, **26** : 2525-2528 (1961).
16. WOODWARD, R. B. and OLAFSON, R. A. — *J. Am. Chem. Soc.*, **83** : 1007 (1961).

17. GILL III, T. J. and MANN, L. T. Jr. — *J. Immunol.*, **96** : 906 (1966).
CARPENTER, C. B., GILL III, T. J. and MANN, L. T. Jr. — *Ibid.*, **98** : 236-250 (1967).
18. PAUSON, P. *et al.* — *J. Chem. Soc.*, 650-656 (1953).
19. SCHLOGL, K. — *Monatsh.*, **88** : 601-621 (1957-1958).
20. RINEHART, K. L., CURBY, R. J. Jr. and SOKOL, P. E. — *J. Am. Chem. Soc.*, **79** : 3420-3424 (1957).
21. FRIEDMAN, E., GILL III, T. J. and DOTY, P. — *J. Am. Chem. Soc.*, **83** : 4050-4053 (1961).
22. DIEHL, H. and SMITH, G. F. — *The Iron Reagents*, G. F. Smith Chemical Co., Columbus, Ohio, 1960, pp. 11-26.
23. GRAHAM, P. J. *et al.* — *J. Am. Chem. Soc.*, **79** : 3416-3418 (1957).