Addition Compounds of Hydrocarbons and *n*-Butyl 3,5-Dinitrobenzoate

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In recent years several addition compounds of the hydrocarbons have been extensively studied.[1] This report describes a new type of hydrocarbon adduct, discovered during a study of the conversion of N-(*n*-butyl)-N-nitroso-3,5-dinitrobenzamide into *n*-butyl 3,5-dinitrobenzoate.[2] The ester from this reaction had the correct melting point, but the melt obtained was slightly turbid (becoming clear at higher temperatures). The infrared spectra and the elementary analyses of these samples were identical with those of the pure ester. The proportion of the high melting form was shown to be unaffected by a large number of the more obvious variables; it was finally determined that traces of stopcock grease were responsible[3] and that the hydrocarbons in the grease had formed addition compounds with the ester. Subsequently, a number of adducts were prepared, most conveniently by the recrystallization of the ester from a sample of the pure hydrocarbon (Table I).

TABLE I

MELTING POINTS OF ADDUCTS

Hydrocarbon	M.p.,[a] °C.	
n-Pentane[c]	(63.6)	64.0–64.5[a]
	(63)	63.5–63.9[b]
n-Heptane	(65)	65.8–66.4[c]
	(63.6)	64.0–65.0[d]
n-Octane	(66)	66.8–67.1[c]
	(63.9)	64.5–66.9[d]
2,2,4-Trimethylpentane[e]	(66)	67.4–68.1[c]
	(63.5)	65.9–67.5[d]
n-Decane	(66.5)	66.9–67.2[f]
n-Hexadecane		69.2–69.6[f]
Methyl oleate	(58)	66.2–68.0
Methyl stearate		62–66[g]

[a] Melting points uncorrected. The same thermometer and apparatus was used for all of the determinations. The rate of heating was *ca.* 0.5° per min.; sinter temp. in parentheses. [b] M.p. in sealed capillary. [c] Melting point determined 10 min. after preparing adduct. [d] Melting point determined 11 hr. after preparing adduct. [e] On one occasion, the pure ester was obtained from this solvent (reason unknown). [f] Adduct held at 0.1 mm. (3 hr.). [g] Adduct prepared from concd. pentane solns. of the components.

All of the normal paraffins tested (with the possible exception of pentane) as well as 2,2,4-trimethylpentane, methyl oleate and methyl stearate formed adducts with *n*-butyl 3,5-dinitrobenzoate.[4] The melting points of the adducts are higher than that of the pure ester (with the exception of the pentane adduct) and increase with the chain length of the hydrocarbon (Table I). The addition compounds of the lower hydrocarbons are rather unstable, that of heptane reverts to the pure ester upon standing at room temperature for one day, whereas the adducts of hydrocarbons such as hexadecane are stable for years under similar conditions. The pure ester can be obtained from the stable adducts either by vacuum sublimation or by recrystallization from ether or pentane.

Analyses of the hexadecane adducts indicate that *ca.* 15 ester molecules are required for each molecule of hexadecane[5] (Table II). The corresponding value of this ratio for octane was *ca.* 11/1 as determined by a weight-loss method. These values are slightly higher than the corresponding ratios found for the urea adducts.[6]

Experimental[7]

Hydrocarbons.—Constant-boiling center cuts of pentane, heptane, 2,2,4-trimethylpentane and octane were used.[8] The decane and hexadecane (kindly furnished by Humphrey–Wilkinson Inc., North Haven, Conn.) and the two esters[8] were used without further purification.

Preparation of *n*-Butyl 3,5-Dinitrobenzoate.—The ester was prepared from *n*-butyl alcohol and 3,5-dinitrobenzoyl chloride in an excess of pyridine. The product was recrystallized from dilute pentane solutions or from concentrated ether solutions to yield a mass of plate-like crystals. The melting points of different preparations differed slightly; however, most samples melted between 63.5 and 64.5° with, on the average, a range of *ca.* 0.5° (lit.[9] 64°).

TABLE II

ANALYSES OF *n*-HEXADECANE ADDUCTS

Ester or adduct	M.p., °C.[a]		Carbon, % Calcd.[d]	Carbon, % Found	Hydrogen, % Calcd.[d]	Hydrogen, % Found	Nitrogen, % Calcd.[d]	Nitrogen, % Found	*e/h*[b]
n-Butyl 3,5-dinitrobenzoate	(63.5)	64.0–64.5	49.26		4.51		10.45		
Adduct after sublimation	(63)	64.0–64.5		49.13		4.44		10.44	
Unwashed adduct		69.2–69.6	60.62	61.10	7.90	7.86	7.11	7.31	1.8[c]
Adduct washed with pentane (−80°)	(69.9)	70.2–70.9	51.28	51.35	5.11	5.05	9.85	9.82	14
Adduct washed with pentane (25°)	(68.5)	69.2–69.6	51.04	51.37	5.04	5.16	9.92	10.06	16

[a] Sinter temp. in parentheses. [b] *e/h* = moles of ester/mole of hydrocarbon. Since the analysis of the ester and the analyses of the adducts are not appreciably different, the values listed for *e/h* are accurate only to *ca.* ±5%. [c] This value is abnormally low because of the film of hydrocarbon on the surface of the crystals. [d] Calcd. for the value of *e/h* given.

Preparation of Adducts.—*n*-Butyl 3,5-dinitrobenzoate (1 part) was dissolved with warming in the hydrocarbon (*ca.* 10 parts by weight); upon cooling, fibrous crystals of the adduct separated. Air was drawn through the filter for *ca.* one minute to remove the excess hydrocarbon from the adducts of the lower hydrocarbons. The excess hydrocarbon

(1) (a) H. M. Powell, *J. Chem. Soc.*, 2658 (1954); (b) J. Monteath Robertson, "Organic Crystals and Molecules," Cornell University Press, Ithaca, N. Y., 1953, pp. 251–255; (c) R. F. Marschner, *Chem. Eng. News*, **33**, 494 (1955).

(2) E. H. White, THIS JOURNAL, **77**, 6011 (1955).

(3) Trace amounts of grease in ordinary laboratory glassware can be detected with *n*-butyl 3,5-dinitrobenzoate. The glassware is rinsed with a solution of the ester in pentane and the solvent is removed *in vacuo*. The ester should now melt, largely at 64°, with distinct turbidity visible to *ca.* 68°.

(4) The fact that hydrocarbon chains of such diverse types can be accommodated suggests that the adducts are not of the cage or clathrate type (footnote 1a), but are probably of the channel type established for urea and thiourea (footnote 1c).

(5) This represents a maximum value, since in removing the surface adsorbed hydrocarbon from the crystals, some of the occluded hydrocarbon may have been removed as well.

(6) O. Redlich, C. M. Gable, A. K. Dunlap and R. W. Millar, THIS JOURNAL, **72**, 4153 (1950).

(7) Melting points uncorrected.

(8) Eastman Kodak Company.

(9) R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1948, p. 226.

was removed from the adducts of the higher-boiling hydrocarbons either by holding the adducts at low pressure or by washing briefly with pentane (*ca.* 5 parts). Using an alternate method, a saturated solution of the ester was prepared in *n*-butyl alcohol and 5% by volume of *n*-hexadecane was added. Crystals appeared only on cooling the solution by 5–10°; these were treated as above, m.p. 68.0–69.5°.

The melting points of the isobutyl, *sec*-butyl, *t*-butyl and *n*-propyl 3,5-dinitrobenzoates and N-(*n*-butyl)-3,5-dinitrobenzamide were not affected by the presence of hydrocarbons; they presumably formed no adducts.

Octane Run.—A solution of *n*-butyl 3,5-dinitrobenzoate (84.5 mg., 0.315) mmole) was prepared in *n*-octane (2.0 g.) and the solution was allowed to evaporate (24°). After 13 hours, the total weight of the reactants had dropped to 87.8 mg.; the subsequent weight loss was rather small, 0.1 mg./day. The ratio by weight of ester to octane in the adduct was, therefore, 84.5/3.3. This is equivalent to 11 moles of ester per mole of octane.

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COMMUNICATIONS TO THE EDITOR

THE RECOVERY OF NORLEUCINE FROM CASEIN AFTER ADMINISTERING NORLEUCINE-3-C^{14} TO INTACT COWS[1]

Sir:

Norleucine has been reported to be a constituent of protein from brain tissue[2] but subsequent work demonstrated that DL-leucine, formed by racemization of L-leucine during protein hydrolysis, and not norleucine had actually been isolated in the original investigations.[3] More recent work has failed to confirm the presence of norleucine in protein.[4]

We injected intravenously two lactating cows with 3.6 millicuries (Cow #941, Trial I) and 3.3 millicuries (Cow #962, Trial II) of DL-norleucine-3-C^{14}, respectively. In Trial I, during 46 hours post-injection, 30% of the Carbon-14 was recovered in milk of which about one-third was present in casein. A casein sample prepared from the milk collected three hours post-injection was hydrolyzed, the amino acids separated on ion exchange resins, crystallized and assayed for C^{14} content according to methods described elsewhere.[5] The non-essential amino acids[6] had high C^{14} levels while the essential amino acids,[6] including leucine and isoleucine, did not contain significant amounts of C^{14}. However, the total C^{14} in the non-essential amino acids accounted for only 52% of the C^{14} in casein. A thorough recheck of all the effluent from the Dowex-50 column near the region of isoleucine–leucine elution revealed a small amount of material with high C^{14} activity that had emerged after leucine. The sample was identified as norleucine by paper chromatographic methods using various solvent systems (phenol, butanol–acetic acid, butanol–benzyl alcohol). We also have recovered and identified norleucine from casein prepared out of the milk collected ten hours after injection of norleucine-3-C^{14}.

We have only preliminary results from Trial II, but these appear to be much the same as results obtained in Trial I. In the casein collected three hours post-injection, the non-essential amino acids accounted for only 59.5% of the Carbon-14 in casein. Norleucine has been identified by C^{14}-measurements on a chromatogram of the casein hydrolysate but has not, as yet, been isolated from the Dowex-50 column.

The presence of norleucine in casein might result from adsorption or co-precipitation although our method for preparing the casein minimized this possibility. The casein was prepared according to our routine procedures by adjusting the *p*H to 4.6 with *N* HCl. The casein was filtered out, washed with water and then redissolved in *N* NH_4OH. This procedure was repeated and the casein, after the third precipitation, was washed with water, followed by alcohol and finally ether. The air dried casein sample was used for the C^{14} study.

To investigate the possibility of norleucine adsorption by casein, 40 microcuries of DL-norleucine-3-C^{14} was added to 1 liter of milk and after standing for 48 hours casein was prepared as described above. Casein samples were taken from the first, second and third precipitation for C^{14}-measurements. There was measurable activity only in the first precipitate and no significant C^{14} in the second or third precipitates. The *in vitro* trial was repeated with essentially the same results. These *in vitro* experiments demonstrate that our method for casein preparation effectively removes adsorbed (or co-precipitated) norleucine.

The results obtained with the intact cow presumably indicate incorporation of norleucine by peptide linkage. We are presently separating peptides from partial casein hydrolysates in an effort to isolate a peptide containing norleucine.

It was important to establish whether norleucine was a natural constituent of casein previously undetected because of low concentration. Control samples included five grams of casein prepared from the milk of one of our cows (Cow #962)[7] and another 5-g. sample of casein purchased from a commercial source. These samples were hydrolyzed, passed over Dowex-50 and the effluent

(1) This investigation was supported by grants from the Atomic Energy Commission and the National Science Foundation.

(2) J. L. W. Thudichum, "Die Chemische Konstitution des Gehirns des Menschen und der Tiere," Franz Pietzcker, Tübingen, 1901. E. Abderhalden and A. Weil, *Hoppe-Seyl. Z.*, **84**, 39 (1913); E. J. Czarnetzky and C. L. A. Schmidt, *J. Biol. Chem.*, **97**, 333 (1932).

(3) R. Consden, A. H. Gordon, A. J. P. Martin, O. Rosenheim and R. L. M. Synge, *Biochem. J.*, **39**, 251 (1945).

(4) M. U. Hassan and D. M. Greenberg, *Arch. Biochem. Biophys.*, **39**, 129 (1952).

(5) A. L. Black and M. Kleiber, *J. Biol. Chem.*, **210**, 895 (1954).

(6) A. L. Black, M. Kleiber and A. H. Smith, *ibid.*, **197**, 365 (1952).

(7) This is the same cow used for Norleucine Trial II but the control milk was collected two years after the injection of Norleucine-3-C^{14}.