SC 2221

Formation of an *S*-glucuronide from tetraethylthiuram disulfide (Antabuse) in man

It has been shown that plant tissues are able to transform *N,N*-dialkyldithiocarbamates into the corresponding glucosides[1] and alanine derivatives[2]. Contrary to plants, microorganisms produce the aminobutyric acid derivatives[3].

Formation of conjugates of dithiocarbamates in the animal organism has not been reported so far. A considerable amount of work has been done on the fate of tetraethylthiuram disulfide (known under a number of trade names such as Antabuse, Refusal, Disulfiram) since the discovery of the remarkable influence on ethanol intoxication symptoms of this dithiocarbamate and related compounds. ELDJARN[4] investigated the metabolism of [^{35}S]Antabuse in man. The analysis of the urine indicated that about 65 % of the ^{35}S is oxidized to sulphate while a small amount, about 6 %, is recovered in the organic-sulphur fraction. MERLEVEDE AND CASIER[5] reported the presence of CS_2 in the expired air of persons during treatment with Antabuse. They were able to recover about 50 % of the administered dose of Antabuse in the form of CS_2. The excretion of unchanged Antabuse in the urine is practically nil, as is stated by several authors[6,7], while the reduced form, diethyldithiocarbamic acid, has been detected in blood and in alkaline urine[7].

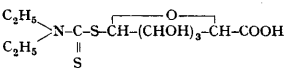
It is evident from this survey of the literature that the greater part of the metabolized Antabuse is excreted as non-conjugated products. The composition of the organic-sulphur fraction remains as yet unknown. The purpose of this investigation was to determine whether or not the presence of conjugates in this fraction could be demonstrated. For the chromatographic detection of conjugates use was made of the catalyzing effect of compounds containing thione groups on the sodium azide–iodine reaction (FEIGL[8]).

Four male persons were given daily 1 g of Antabuse for 3 successive days. During this time and for 3 days afterwards the urine was collected and preserved under toluene. The combined urine (27 l) was subsequently concentrated *in vacuo* to a volume of 2 l and freed from the precipitate formed. The filtrate was acidified with 250 ml of glacial acetic acid and extracted 3 times with an equal volume of butanol. The precipitate as well as the water layer contained besides cystine no other catalytically active compounds. The combined butanol extracts were concentrated *in vacuo* to a volume of 300 ml and subjected in 3 portions to a 100-cycles counter-current fractionation between butanol–acetic acid–water (4:1:5, v/v/v) in a Craig apparatus. Most of the hydrophilic materials, mainly urea and salts, remained in the first 40 tubes. The fractions in the Tubes 53–66 catalyzed the iodine–sodium azide reaction while also the Fractions 95–100 gave a positive, though very weak, response. The latter fractions probably contained some unchanged Antabuse. The Fractions 53–66 were concentrated and gave 10 g of a syrup. A subsequent counter-current distribution between butanol and water reduced its weight to 5 g. Paper chromatography revealed one positive spot with $R_F = 0.26$ in propanol–water (85:15, v/v) and $R_F = 0.63$ in butanol–acetic acid–water (4:1:1, v/v/v) while the eluted spot gave a positive reaction for hexuronic acids with naphthoresorcinol–H_2SO_4. Moreover, paper ionophoresis in phosphate buffer (pH 7.0) showed the compound to be an acid. These facts suggested that the compound was the *S*-glucuronide of diethyldithiocarbamic acid. The supposition could be confirmed by synthesis of this compound. The latter

was prepared at our institute by Dr. PLUIJGERS and Mr. BERG from methyl-2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-α-D-glucuronate and sodium diethyldithiocarbamate followed by saponification of the triacetyl methyl ester thus obtained. Details of this synthesis will be published elsewhere.

Definite proof of the identity of the supposed glucuronide could be obtained by isolation of its triacetyl methyl ester by a procedure essentially according to KAMIL *et al.*[9]. For this purpose the above-mentioned 5 g crude product was dissolved in water and the solution adjusted to pH 8 with ammonia. Basic lead acetate was added in excess, the precipitate centrifuged off and decomposed with H_2S. Concentration of the filtrate yielded 2.5 g of a still very impure product. The acid was then methylated with diazomethane and the reaction product subjected to a 100-cycles counter-current distribution between ethyl acetate and water. The Feigl-positive Fractions 50–59 gave, after evaporation of the solvents, 390 mg of a gum, which was consequently acetylated with pyridine and acetic anhydride. Finally the compound could be crystallized from boiling cyclohexane. Yield: 280 mg. The compound had m.p. 137° (uncorr.). (Found: C, 46.61; H, 5.77; N, 3.04; S, 13.91. $C_{18}H_{27}NO_9S_2$ requires: C, 46.40; H, 5.85; N, 3.01; S, 13.76 %.)

Melting points and infrared spectra of the isolated derivative and the synthetic triacetyl methyl ester proved to be identical. Thus, the conjugate formed from Antabuse is *N*,*N*-diethylthiocarbamoyl 1-thio-β-D-glucopyranosiduronic acid:



The quantity of the isolated compound accounted for 0.75 % of the administered dose of Antabuse. Of course the actual percentage should have been higher if not for the inevitable losses during the purification. Moreover, the collection of urine was limited to only 3 days after the last administration of Antabuse.

It remains obscure whether or not the glucuronide plays a role in the Antabuse–ethanol reaction. It is true that oral administration of 1.3 g of the glucuronide —an amount equivalent to 0.6 g of Antabuse—to an adult caused the appearance of the typical Antabuse-induced sensitization to ethanol, but its action may be due to the aglycone.

Formation of a glucuronide from Antabuse presumably is not restricted to man. KAMIL *et al.*[10] observed an increased output of total (free plus conjugated) glucuronic acid in the urine of rabbits during treatment with Antabuse. It seems reasonable to suggest that also in this case the *S*-glucuronide was responsible for the increase found.

Formation of *S*-glucuronides *in vivo* has been reported earlier in two cases; PARKE[11] partially purified a glucuronide formed from thiophenol in rabbits while CLAPP[12] reported the isolation of the *S*-glucuronide of 2-mercaptobenzothiazole from the urine of dogs treated with 2-benzothiazolesulfonamide.

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Received November 29th, 1962

SC 2234

The isolation of squalene from human brain

In 1959 NICHOLAS AND THOMAS[1] gave evidence for the presence of squalene in rat brain after injecting intracerebrally sodium [2-^{14}C]mevalonate into the animal. More recently, HONEGGER[2] indicated its presence in brain tissue of multiple-sclerosis patients and normals by means of thin-layer chromatography. However, additional proof of identity remained to be done. JATZKEWITZ AND MEHL[3] mention also this hydrocarbon as a brain substance.

In this paper we give a report on the isolation and identification of squalene from human brain. Adult human brains were obtained *post-mortem* as soon as possible after death from cases in which there had been no pathological changes.

Two whole brains (about 2.5 kg) were freed from blood and meninges under running tap-water, minced in a Waring blendor and exhaustively extracted with cold acetone. The combined extracts were evaporated *in vacuo* to dryness and the residue crystallized from methanol. In this manner a great part of cholesterol was removed from a mixture. The filtrate was freed of solvent *in vacuo*, the semicrystalline residue dissolved in light petroleum (30–50°) and chromatographed on activated alumina. Each fraction was tested for squalene by paper chromatography. The light petroleum eluates (Fractions 1–6) showed a negative reaction and were discarded. The first benzene eluates (Fractions 7–12) were squalene positive and gave a viscous oil (880 mg) which still contained a considerable quantity of cholesterol. Therefore, it was dissolved in hexane and rechromatographed on Florisil (60/100 mesh, Floridin Company, Tallahassee, Florida) using the technique described recently by CARROLL[4]. A typical experiment is presented in Fig. 1. A sample of 92 mg of the oily product was put on 12-g columns of Florisil. An unidentified substance or mixture of substances (Area 1), squalene (Area 2) and cholesterol (Area 3) were successively eluted with hexane (30 ml), 5% diethyl ether in hexane (30 ml), 15% diethyl ether in hexane (25 ml) and 25% diethyl ether in hexane (25 ml) respectively. Only Fractions 8–13 were squalene positive. Approx. 13 mg of the highly purified hydrocarbon were isolated from this run. Thus, the clean-cut separation of squalene from other lipide classes could be effected.