STEROLS OF THE HOLOTHURIANS Chiridota discolor and Synallactes chuni

P. S. Dmitrenok, L. K. Shubina,
T. N. Makar'eva, and V. A. Stonik

UDC 547.925:593.96

Steroid metabolites from holothurians of the family Chiridotidae (order Apoda) and Synallactidae (order Aspidochirota), have not been studied previously.

We have investigated fractions of the free steroids of Chiridota discolor and Synallactes chuni (determined by A. V. Smirnov) collected during the second expedition voyage of the Scientific Research Vessel "Akademik Oparin" in August, 1986, in the Sea of Okhotsk at depths of 128 and 430 m, respectively.

Ethanolic extracts from the tissues of the holothurians were concentrated in vacuum. Column chromatography of the residues on polikhrom-1 (water–ethanol) and then on silica gel L (hexane–ethyl acetate (6:1) gave the crude total free sterols, which were recrystallized from ethanol. Then part of the free sterols was acetylated with acetic anhydride in pyridine (1:1). The acetates of the sterols from C. discolor were separated into fractions by preparative TLC (hexane–benzene (4:1)) on silica gel impregnated with silver nitrate.

The combined free steroids and acetates from both holothurians, and also the fractions of the acetates obtained from C. discolor were analyzed with the aid of capillary gas-liquid chromatography on a Perkin-Elmer Sigma 2000 chromatograph with a 0.2 mm × 25 m column containing OV-101 at a temperature of 290°C with argon as the carrier gas, and by chromato-mass spectrometry on a LKB-9021 with a 0.32 mm × 25 m capillary column containing SE-54 at temperatures of 200-240°C (5°C/min) and 240-260°C (0.5°C/min) at an ionizing energy of 70 eV, the carrier gas being helium.

The steroid components were identified by their mass spectra in combination with their chromatographic behavior in capillary GLC.

TABLE 1. Composition of the Steroid Fractions of the Holothurians, %

Sterol	C. discolor	S. chuni	RRT (relative to cholesterol)	M^+ of the acetate
$C_{26}\Delta^{22}$	0,15	0,14	0,73	414
$C_{26}\Delta^{22}$	0,26	0,18	0,74	414
$C_{26}\Delta^{7,22}$	0,41	0,27	0,78	412
$C_{26}\Delta^{0}$	—	0,10	0,81	416
$C_{27}\Delta^{22}$	1,12	0,96	0,92	428
$C_{27}\Delta^{7,22}$	Traces	Traces	0,99	426
$C_{27}\Delta^{5}$	1,50	3,10	1,00	—
$C_{27}\Delta^{0}$	7,60	5,20	1,02	430
$C_{27}\Delta^{7}$	5,00	—	1,10	428
$C_{28}\Delta^{22}$	4,10	12,25	1,11	442
$C_{28}\Delta^{7,22}$	5,53	15,02	1,20	440
$C_{28}\Delta^{24(28)}$	2,65	1,95	1,24	442
$C_{28}\Delta^{0}$	2,06	1,98	1,26	444
$C_{28}\Delta^{7,24(28)}$	2,58	—	1,34	440
$C_{28}\Delta^{7}$	3,21	Traces	1,36	442
$C_{29}\Delta^{22}$	—	4,33	1,33	456
$C_{29}\Delta^{22}$	—	1,90	1,35	456
$C_{29}\Delta^{7,22}$	4,39	7,14	1,45	454
$C_{29}\Delta^{0}$	6,78	5,94	1,50	458
$C_{29}\Delta^{24(28)}$	Traces	Traces	1,60	456
$C_{29}\Delta^{7}$	37,40	33,0	1,62	456
$C_{29}\Delta^{7,24(28)}$	3,50	—	1,65	454
$C_{30}\Delta^{7,24(28)}$	1,71	—	1,77	468
$C_{30}\Delta^{7}$	3,90	—	1,85	470
$C_{30}\Delta^{7,24(28)}$	1,95	—	1,89	468

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, USSR Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 303-304, March-April, 1988. Original article submitted July 10, 1987; revision submitted October 27, 1987.

0009-3130/88/2402-0261$12.50

A total of 22 components were identified in C. discolor, and 19 in S. chuni. The results of the analyses are given in Table 1.

The fractions studied contained as the main component 24-ethylcholest-7-en-3β-ol and were characterized by high amounts of $\Delta^{7,22}$-sterols and stannols. With respect to their sterol compositions, C. discolor and S. chuni did not differ essentially from the majority of other animals of this class. All the compounds identified, with the exception of the $C_{26}\Delta^{0}$ [1] and $C_{30}\Delta^{7}$ [2] compounds, have been detected previously in holothurians [2-4].

LITERATURE CITED

1. C. Delseth, L. Tolela, P. J. Sheuer, R. J. Wells, and C. Djerassi, Helv. Chim. Acta, 62, 101 (1979).
2. L. J. Goad, in: Marine Natural Products (ed. P. J. Sheuer), Academic Press, Vol. 2 (1978), p. 76.
3. J. A. Ballantine and A. Lavis, J. Exp. Mar. Biol. Ecol., 53, 89 (1981).
4. N. I. Kalinovskaya, T. A. Kuznetsova, and G. B. Elyakov, Comp. Biochem. Physiol., 74B, 597 (1983).

SPECTROPHOTOMETRIC DETERMINATION OF DEOXYCORTICOSTERONE ACETATE

D. I. Dochinets, B. P. Zorya, and V. V. Petrenko

UDC 615.272.2.07:535.243

Deoxycorticosterone is a mineralocorticosteroid the source of which is either the adrenal glands of slaughtered cattle or natural substances of steroid structure, especially cholesterol. In medicine, deoxycorticosterone is used mainly in the form of the acetate (DOCSA) for the regulation of the mineral metabolism; it increases the tonus and improves the working efficiency of muscles [1].

In spite of its wide use, the analysis of DOCSA has been inadequately developed. The methods that have been described for the photocolorimetric [2-4] and spectrophotometric [2, 5] determination of this drug are characterized by low sensitivity, inconveniences in performance, and lengthiness.

Our aim was to develop a highly sensitive procedure, simple in performance, for the quantitative determination of DOCSA. The proposed procedure is based on the reaction with isatin hydrazone. It has been established that DOCSA interacts with isatin hydrazone in dioxane with the formation of a yellow product. The intensity of the coloration is directly proportional to the amount of DOCSA in the sample under investigation and obeys Beer's law within the range of concentrations of 1.2-3.6 mg of substance in 100 ml of solution. The reaction was performed at the temperature of the boiling water bath with the use of a 1% solution of isatin hydrazone. Dioxane of ch.d.a. ["pure for anlaysis"] grade was used as the solvent for the reagent and for the compound to be determined. It is assumed that a hydrazone is formed during the reaction.

The reaction that we have developed has the following spectral characteristics: absorption maximum 445 nm; molar absorption coefficient 10,800; specific absorption 0.02899 $cm^1/\mu g$; Sandell's coefficient 0.03449; limit of detection 1.72 μg/ml.

The quantitative determination of DOCSA was carried out in the following way. An accurately weighed sample (0.015-0.044 g) of the substance was dissolved in dioxane in a 50-ml measuring flask, and solvent was added to the mark. To 1 ml of the resulting solution in test-tube was added 5 ml of a 1% solution of isatin hydrazone and one drop of 5% HCl. The test-tube was placed in the boiling water bath for 10 min and was cooled, and the contents were transferred quantitatively to a 25-ml measuring flask and made up to the mark with dioxane. In parallel under the same conditions, an experiment with a standard sample of DOCSA (0.0300 g in 50 ml of dioxane) and one with a solution of the background were performed. The optical densities of the colored solutions were measured relative to the background on a SF-26 spectrophotometer at 445 nm using a cell with a layer thickness of 1 cm.

Zaporozh'e Medical Institute.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 305-306, March-April, 1988. Original article submitted September 10, 1987.

0009-3130/88/2402-0262$12.50