12β,25-Di-O-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammaran-3α-ol (VIII). $[\alpha]_D^{20}$ −21.2° (c 0.1; pyridine), $C_{42}H_{72}O_{14}$.

3α,12β,25-Tri-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammarane (X). $[\alpha]_D^{20}$ −21.4° (c 0.8; pyridine), $C_{48}H_{82}O_{19}\cdot 3H_2O$.

3β-12β,25-Tri-β-D-glucopyranosyloxy-20(S),24(R)-epoxydammarane (XI). $[\alpha]_D^{20}$ −20.8 (c 1.0; pyridine), $C_{48}H_{82}O_{19}\cdot 3H_2O$.

SUMMARY

The 12,25-di-O- and 3,12,25-tri-O-glucosides of 20(S),24(R)-epoxydammarane-3α,12β,25-triol and of its 3-epimer have been obtained under the conditions of the orthoester method of glycosilation and by Helferich's modification.

LITERATURE CITED

1. Y. Nagai, O. Tanaka, and S. Shibata, Tetrahedron, 27, 881 (1971).
2. G. B. Elyakov and Yu. S. Ovodov, Khim. Prir. Soedin., 699 (1972).
3. O. Tanaka and S. Yahara, Phytochem., 17, 1353 (1978).
4. L. N. Atopkina and N. I. Uvarova, Khim. Prir. Soedin., 205 (1980).
5. N. K. Kochetkov, A. F. Bochkov, T. A. Sokolovskaya, and N. I. Snyatkova, Carbohydr. Res., 16, 17 (1971).
6. B. Helferich and K. Weis, Chem. Ber., 89, 314 (1956).
7. N. Nagai, N. Tanaka, and S. Ishikawa, Chem. Pharm. Bull., 19, 2061 (1973).

ANALYSIS OF AN EXTRACT OF *Atropa belladonna* BY CENTRIFUGAL COLUMN CHROMATOGRAPHY

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The possibility has been shown of using the method of centrifugal column chromatography to accelerate the process of separating medicinal substances in mixtures.

The development of methods for analyzing mixtures of medicinal substances is associated with the development of fast, simpler, and improved methods of separating the initial mixtures. One of such methods is centrifugal column chromatography. In this process, the speed of separation of multicomponent medicinal preparations depends on the rate of passage of the extractant through the column. The separation can be accelerated by feeding the extractant to the column under a pressure created with the aid of complex and expensive pumping systems or with the aid of centrifugal forces.

It must be mentioned that the method of separation on columns under the action of centrifugal forces has not been used to solve the problems with which pharmaceutical analysis is faced. The method developed has previously been used for separation with the subsequent determination of steroid hormones [1], sterols and fatty acids [2], amino acids [3], pigments [4], carbohydrates [5], blood components [6], anthraquinone derivatives [7], etc. Investigations performed have shown undoubted advantages of centrifugal column chromatography in comparison with ordinary column chromatography, such as speed of analysis, small amounts of the initial substances and reagents required, and simplicity of the apparatus used.

The separation of medicinal substances in mixtures is carried out in centrifugal fields on a TsLK-1 centrifuge having three strictly fixed speeds: 1000, 1500, and 3000 revolutions per minute. In view of the fact that under the action of a high centrifugal force creating a pressure above 100 atm some adsorbents undergo extreme self-compaction, we have created a scheme using a laboratory autotransformer. The autotransformer ensures a variation in the

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voltage of from 0 to 220 V, which permits the speed of the centrifuge to be decreased. It has been established that the optimum speeds of rotation of the centrifuge are between 500 and 1000 rpm.

The analysis of mixtures of medicinal substances has been performed in 5 × 75 mm glass columns filled with the sorbent by the dry method. The sorbent used was type L-100/250 silica gel (1 g of powder for each column).

Method of Determination. About 15 g of dry belladonna extract (accurately weighed) is placed in a separatory funnel, 15% of a 5% solution of ammonia is added, and the mixture is shaken for 15 min. The sum of the alkaloid bases is extracted with ether (4 × 5 ml) and then with chloroform (4 × 5 ml), and the extracts are combined. The completeness of extraction of the total alkaloids is checked by thin-layer chromatography on Silufol UV-254 plates in the acetone–25% ammonia (95:5) system. After the solvents have been distilled off, the dry residue is dissolved in 5 ml of 96% ethanol.

Each of two columns is charged with 1 g of L-100/250 silica gel. On one column is deposited 0.5 ml of the ethanolic solution of the mixture of alkaloids to be analyzed. The alkaloids are eluted from the column with a mixture of acetone and 25% ammonia (95:5) in 1-ml portions. The eluate from the first column is transferred to the second. In view of the fact that the apoatropine present in the total bases in very small amount is extracted from the columns in the first two portions of eluate, they must be discarded on quantitative determination. The following six portions of eluate contain the scopolamine. After evaporation of the eluate, the dry residue is dissolved in 5 ml of 0.1 N sulfuric acid. The optical density of the solution is measured on a spectrophotometer at a wavelength of 259 nm [8]. The atropine remaining in the column is eluted with the same extractant in six 1-ml portions. The eluate is evaporated and the dry residue is dissolved in 10 ml of 0.1 N sulfuric acid. The optical density of the solution is measured at a wavelength of 258.5 nm. The comparison solutions for the two substances consist of 0.1 N sulfuric acid [9]. Solutions of scopolamine hydrobromide and of atropine sulfate in a concentration of 900 μg/ml in 0.1 N sulfuric acid are used as standards.

In the spectrophotometric determination of the alkaloids, after their separation by centrifugal column chromatography errors of ±2.35% (scopolamine) and ±4.04% (atropine) have been obtained, while by the method recommended by the State Pharmacopeia of the USSR (Xth edn.), the error of the determination amounts to ±2.44%, calculated as hyoscyamine:

Substance	$\bar{X}$	S	$S_{\bar{X}}$	ε_{rel}	ε_{abs}	A
Scopolamine	95.42	2.53	0.96	2.46	2.35	95.42 ± 2.35
Atropine	93.28	4.36	1.65	4.33	4.04	93.28 ± 4.04
Belladonna extract	93.30	2.63	0.99	2.62	2.44	93.30 ± 2.44

As can be seen, the error of the determination in the analysis of substances by the proposed method and the official method differ insignificantly, while our method also permits the determination of individual alkaloids present in the extract.

The method of analyzing belladonna extract using centrifugal column chromatography that we have developed makes it possible to shorten the time of analysis 4- to 6-fold in comparison with the method recommended by the State Pharmacopeia of the USSR (Xth edn.).

SUMMARY

1. The possibility has been shown of using the method of centrifugal column chromatography for accelerating the process of separating medicinal substances in mixtures.

2. A method has been developed for isolating the two main alkaloids from *Atropa belladonna* with their subsequent determination which permits the time of analysis to be shortened by a factor of 4-6.

LITERATURE CITED

1. F. W. Karasek, Res. Dev., 21, No. 7, 43 (1970).
2. J. Meijer, M. Eid, and P. Verduin, Z. Anal. Chem., 236, No. 6, 426 (1968).
3. Y. Ito and R. Bowman, Science, 173, No. 3995, 420 (1971).
4. H. Pfander, Chromatographia, 9, No. 72, 630 (1976).
5. C. Scott and W. Pitt, Am. J. Clin. Pathol., 53, No. 5, 701 (1970).

6. C. Scott and J. Mailen, Clin. Chem., 18, No. 8, 749 (1972).
7. I. P. Shevtsovich, Zavodsk. Lab., No. 24, 502 (1963).
8. Yu. V. Shostenko, I. S. Simon, T. A. Pletneva, and T. N. Gubina, Khim.-Farm. Zh. No. 7, 46-49 (1969).
9. State Pharmacopeia of the USSR [in Russian], Moscow, Xth edn. (1968), p. 254.

CRYSTAL AND MOLECULAR STRUCTURE OF THE DITERPENE ALKALOID TALATISINE

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As a result of x-ray studies, the spatial structure and conformation of the talatisine molecule have been determined. The mean bond lengths are C—C 1.539(6) Å, N—C 1.493(5) Å, HO—C(sp^3) 1.429(5) Å. The six-membered rings A and B have the chair conformation, and rings C and D have distorted boat conformations. The five-membered rings E, F, and G have the envelope conformation.

The alkaloid talatisine has been isolated from the roots of the plant *Aconitum talassicum* M. Pop. [1] collected in the mountains of the Talas Alatau. In order to determine the spatial structure of the diterpene alkaloid talatisine, we have performed an x-ray study.

The conformation of the talatisine molecule is shown in Fig. 1. It has a rigid three-dimensional skeleton consisting of seven rings. The conformations of the rings can be judged from the figures of Table 1. The cyclohexane ring A (the C(1), C(2), C(3), C(4), C(5), and C(10) atoms) is a $^{10}C_3$ chair close to the ideal form with the C(3) and C(10) atoms deviating in opposite directions from the main plane of the other four atoms by 0.60 and 0.61 Å. The six-membered ring B (the C(5), C(6), C(7), C(8), C(9), and C(10) atoms) is a distorted $^6C_9$ chair with the C(6) and C(9) atoms deviating in opposite directions by 0.65 and —0.86 Å. Ring C (the C(8), C(9), C(11), C(12), C(15), and C(16) atoms) is a distorted $^{8,12}B$ boat; the deviations of the C(8) and C(12) atoms are almost 0.65 and 0.69 Å, respectively, but the other members of the ring deviate from the mean plane by ±0.12 Å, and ring D (the C(8), C(9), C(11), C(12), C(13), and C(14) atoms) likewise has the $B_{8,12}$ boat conformation, the C(8), and C(12) atoms deviating in different directions by —0.62 and —0.75 Å. The five-membered rings E (the C(5), C(6), C(10), C(20), and N atoms) and F (the C(4) C(5), C(6), and C(19), and N atoms) have a ^6E envelope conformation differing somewhat from the ideal [2], the deviations of the C(6) atoms being, respectively, —0.886 Å and —0.87 Å, and ring G (the C(8), C(9), C(10), C(14), and C(20) atoms) is an almost ideal ^8E envelope, the deviation of the C(8) atom amounting to 0.78 Å.

The bond lengths and valence angles are given in Tables 2 and 3. The lengths of the ordinary C—C bonds in the rings vary from 1.496(5) to 1.596(6) Å, but the mean value of 1.539(6) Å coincides with the standard 1.541(3) [3], and also with the value for alkaloids [4, 5]. The mean length of the N—C bonds is 1.493(5) Å, i.e., it almost coincides with the value of 1.50 Å for a protonated quaternary nitrogen $H—\overset{+}{N}[C(sp^3)]_3$ [6], and in the diterpene alkaloids hypognavinol [4] and anhydrohypognavinol [5] this value is 1.53 Å. The mean length of the HO—C (sp^3) bonds is 1.429 (5) Å, fairly close to the usual values [3-5].

The sizes of the valence angles in the talatisine molecule vary: in the five-membered rings from 95 to 110°, and in the six-membered rings from 107 to 117°. In spite of the considerable variations in the valence angles, the values agree with those given for organic compounds [7]. The deviations of the rings from the ideal conformations, and also the variations in bond lengths and valence angles that have been mentioned are due to some overall strain of the talatisine molecule. The nature of the variation in the bond lengths for the hydrogen atoms is given in Table 4. The O(2)H and O(3)H hydroxy groups are located on the same side of the plane of ring C and form a O(2)•••O(3) intramolecular hydrogen bond with a length of 2.88 Å.

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